There is, however, significant practice variability, with such examination becoming increasingly uncommon in routine practice. This has been attributed to a lack of consensus on when to evaluate the CCS, a lack of data on pathogenicity of findings, and a perception of low diagnostic yield. In retrospective review, we sought to examine the rationale for CCS examination in a large blended autopsy practice and evaluate the overall diagnostic yield.
Design: Archival databases of the Mayo Clinic Tissue Registry (Rochester, MN) were searched for cases in which the cardiac conduction system was evaluated. Reasons for evaluation, demography and histopathologic findings were recorded. Whether or not the findings were deemed contributory to cause of death was also noted.
Results: Of the 9290 complete autopsies performed, 215 cases (2.3%) had CCS examination. Mean age was 53.9 yrs (range 0.2-98 yrs) and 127(59.1%) were men. There were 43(0.5%) cases of sudden death without an anatomic substrate, of which 22 were infants and 9 were epileptic deaths. Of the remaining 12 cases of undetermined death, 9(75%) had CCS examination, 2 of which had pathology believed to contribute to death. There were 79 cases where the CCS was submitted because of clinical history of arrhythmia or systemic disease that may involve the CCS. For this cohort, 37(46.8%) cases had CCS pathology believed to contribute to death. In the remaining 125 cases, the reason for submission was primarily educational. In this last cohort, 8(6.4%) cases had pathology that contributed to death. The most frequently encountered incidental finding was fibromuscular dysplasia of the AV nodal artery.
Conclusions: This represents the largest retrospective analyses of examination of the CCS to date. Determining whether histopathology of the CCS is likely to help in determination of cause of death can be guided by the clinical circumstances, with the highest yield in sudden unexpected adult deaths. Interestingly and unexpectedly, however, incidental findings within the CCS that likely contribute to the cause of death can be encountered with some frequency. More routine and widespread examination of the conduction system is necessary to help determine significance of findings and their overall bioepidemiology.

Cytopathology

314 Multi-Institutional Study of Fine Needle Aspiration for Thyroid Lymphoma

Laura Adhikari, Jordan Reynolds, Paul Wakely. Washington University School of Medicine, St. Louis, MO; Cleveland Clinic Foundation, Cleveland, OH; Wexner Medical Center, Ohio State University, Columbus, OH.
Background: Primary thyroid lymphoma is a rare neoplasm accounting for 1-5% of thyroid malignancies. Although thyroid fine needle aspiration (FNA) biopsy is currently an extremely common procedure, little information exists on the accuracy and morphology of FNA cytopathology in thyroid lymphoma.
Design: Pathology databases at Washington University in St. Louis, Wexner Medical Center, and the Cleveland Clinic Foundation were searched for thyroid FNA biopsies having a diagnosis of lymphoma or atypical lymphoproliferative cells, or a corresponding tissue diagnosis of thyroid lymphoma having a prior FNA biopsy.
Results: Sixty-eight cases were retrieved from 64 patients from three institutions; 67 cases with histologic confirmation. Forty-six specimens were from women (68%), ages 21 - 87 years (mean= 60). A great majority of aspirates were surgically confirmed diffuse large B-cell lymphoma (DLBL) (n=43), followed by classical Hodgkin lymphoma (5), chronic lymphocytic leukemia (5), high grade non-Hodgkin lymphoma (3), follicular lymphoma (2) and single cases each of mantle cell lymphoma, Burkitt lymphoma, 'double-hit' lymphoma, mucosa-associated marginal zone B-cell lymphoma, low grade non-Hodgkin lymphoma, and a plasma cell neoplasm. Fifty-one cases were in patients with no prior history of lymphoma. Light chain restriction was detected in 34 specimens (by FCM in 32 cases or PCR, 2 cases). FCM was polyclonal (n=7) or inconclusive (2) with a 25 cases not having FCM performed or not having enough viable cells for evaluation. Four cases showed lymphocytic thyroiditis on surgical follow-up with 2 of these cases having a small monoclonal lymphoid population detected by flow cytometry. Forty-seven aspirates were diagnosed as lymphoma (n=29) or suspicious (n=18) for lymphoma (sensitivity 73%), 11 atypical, 7 benign, 2 unsatisfactory, and 1 suspicious for carcinoma.
Conclusions: An FNA diagnosis of lymphoma or suspicious for lymphoma was most often encountered in women, and was possible in over half of our cases. DLBL was the most common form of thyroidal lymphoma in this series (63%). 7% of aspirates were mistakenly interpreted as lymphocytic thyroiditis.

315 Lower Endoscopic Ultrasound-Guided Fine-Needle Aspiration: A Useful Diagnostic Tool for Perirectal and Rectal Lesions – A Large Series in a Single Tertiary Referral Hospital

Roula Albadine, Geneviève Soucy, Bich Nguyen, Sarto Paquin, Anand Sahai, Gille Gariepy. CHUM St Luc, Montréal, QC, Canada.
Background: Lower Endoscopic Ultrasound-Guided Fine-Needle Aspiration (LEUS-FNA) of rectal /perirectal lesions is a safe, minimally invasive, and well tolerated procedure that provides valuable information which affects patient management. EUS-FNA is useful to demonstrate or rule out cancer recurrence in patient with perirectal lesions and a history of cancer (evidence level 2+). Herein, we presented our experience of LEUS-FNA to evaluate pelvic (rectal/perirectal) lesions.
Design: LEUS-FNAs were retrieved from the archives of our University Hospital, from 2001- 2014. The cytopathology findings, corresponding histology, immunohistochemistry, and clinical data were collected. The sensitivity and specificity of LEUS-FNA were calculated in a subset of patients with available surgical pathology.
Results: 114 specimens were retrieved. Histopathology materiel obtained after LEUS-FNA were available in 37 patients. Masses measured 5–100 mm (mean: 27.5 mm) in diameter. Recurrent cancer was clinically suspected in 46% of cases (n=53). The aspirated material showed malignant (n=48), benign (n=41), atypical/suspicious (n=6) and nondiagnostic cytology (n=19). Malignant cases were adenocarcinoma (n=24), neuroendocrine tumor (n=3), squamous cell carcinoma (n=5), urothelial carcinoma (n=2), positive for malignant cells NOS (n=12), gastrointestinal stromal tumor (n=1) and non Hodgkin lymphoma (n=1). The benign cytology cases were negative for malignant cells (n=32), schwannoma (n=1), and 8 non neoplastic lesions including: abscess (n=3), endometriosis (n=2), hematoma (n=1), malacoplakia (n=1) and mucinous cyst (n=1). Histology confirmed 11/12 negative cytology; one false negative cytology of lymph node. Compared to surgical pathology, LEUS-FNA showed 91% sensitivity, 100% specificity, with diagnostic accuracy of 95%, a positive predictive value of 100% and a negative predictive value of 88%. Discrepancies were likely due to cytology sampling errors.
Conclusions: Lower EUS-FNA has an excellent diagnostic accuracy for lesions in the gut wall and surrounding tissues; LEUS-FNA has a high diagnostic accuracy for preoperative staging for rectal cancer and for early detection of recurrent local disease. Further more, LEUS-FNA allows cytological examination and ancillary studies (immunohistochemistry, flow cytometry), that are helpful for accurate determination of nature of pelvic masses in patient without a history of cancer.

316 Combining the Most Commonly Used Immunomarkers—TTF-1, Napsin A, CK7, CK5/6, and P63—in the Subclassification of Primary and Metastatic Non-Small Cell Lung Carcinoma (NSCLC): A Retrospective Study of 246 Fine Needle Aspiration Cases

Derek Allison, Yuting Wang, Li Chen, Susan Geddes, Frederic Askin, Edward Gabrielson, Qing Kay Li. Johns Hopkins University School of Medicine, Baltimore, MD; Magdalen College, University of Oxford, Oxford, United Kingdom.
Background: Fine needle aspiration (FNA) biopsy plays a critical role in the diagnosis and staging of non-small cell lung carcinomas (NSCLC). The accurate subclassification of NSCLC into adenocarcinoma (ADC) and squamous cell carcinoma (SqCC) is crucial for targeted therapy. FNA specimens, which are often small and contain artifacts, pose a diagnostic challenge in distinguishing between ADC and SqCC. Although prior studies have addressed the performance of individual diagnostic markers, the evidence-based utility of these markers in combination has yet to be determined. Therefore, a restrospective study was performed to evaluate the utility of TTF-1, Napsin A, CK7, P63, and CK5/6, individually and in combination to subclassify primary and metastatic NSCLCs by FNA.
Design: A total of 246 FNA cases comprised of 102 primary NSCLC and 144 primary NSCLC metastases were identified by a medical record search over a two year period. The immunostaining patterns of TTF-1, Napsin A, CK7, P63 and CK5/6 were correlated with the morphological diagnosis of the tumor. The Bootstrap re-sampling approach was used to analyze the performance of individual markers and the combination of individual markers. A P value less than 0.05 was considered statistically significant.
Results: In 72 primary ADCs, TTF-1, Napsin A, and CK7 showed a sensitivity/specificity of 84.5%/96.4%, 92.0%/100%, and 93.8%/50.0%, respectively. In 30 primary SqCCs, P63 and CK5/6 showed a sensitivity/specificity of 91.7%/78.3% and 100%/77.8%, respectively. In 131 metastatic ADCs, Napsin A showed a significantly higher specificity (100%) than TTF-1 (87.5%) and CK7 (25%). In 13 metastatic SqCCs, CK5/6 showed a significantly higher specificty (84.6%) than P63 (68.4%). The combination of markers demonstrated an improved sensitivity and specificity in certain cases; however, TTF-1 and Napsin A in ADCs and CK5/6 in SqCCs performed acceptably individually.
Conclusions: This study demonstrates that TTF-1, Napsin A, CK7, P63 and CK5/6 have variable sensitivity and specificity in the subclassification of NSCLCs. Further, both sensitivity and specificity were increased by combining the interpretation of these individual markers in certain cases. Based on these results, it is recommended to use an algorithmic approach combining TTF-1, Napsin A, and CK7 alongside p63 and/or CK5/6 to subclassify NSCLCs by FNA.

317 Poor Cell Block Adequacy Rate for Molecular Testing Improved With the Addition of Diff Quik Stained Smears: Need for Better Cell Block Processing

Evan Alston, Jessica Levesque, Emmanuel Agosto-Arroyo, Karen Janowski, Gina Coshatt, Shuko Harada, Isam Eltoum. University of Alabama, Birmingham, AL.
Background: As molecular assays improve and the number of targeted therapeutic agents increases, the clinical demand for ancillary molecular testing on small samples, including cytologic specimens, will continue to grow. Fine needle aspiration provides a minimally invasive source of tumor cells for molecular analysis, and this testing is typically performed on the paraffin-embedded cell block. However, there is currently a lack of standardization in cytologic specimen preservation as well as cell block preparation, and this may account for the fact that a substantial number of cell blocks contain a quantity of tumor cells insufficient for molecular analysis. The objective of this study is to assess the frequency and type of samples, including both surgical biopsies and cytologic material, deemed unsatisfactory for molecular testing in a large academic institution.
Design: A retrospective search of the laboratory information system was performed for all cases submitted for EGFR, KRAS, or ROS-1 molecular testing from Sep 2013 to Aug 2014. These cases included both primary and metastatic lesions of pulmonary adenocarcinoma, poorly differentiated non-small cell carcinoma, and large cell neuroendocrine carcinoma. The specimens consisted of surgical biopsies as well as cytologic material. The number of specimens deemed unsatisfactory for analysis was compared across four specimen categories: large biopsy, small biopsy, cell block alone, and cell block with air-dried Diff Quik stained (DQ) smears.

Results: 176 cases were studied, of which 42 (23.86%) were unsatisfactory. Specimens were deemed unsatisfactory for analysis due to DNA degradation, inadequate specimen decalcification, or an insufficient number of tumor cells. 5/72 (6.94%) large surgical biopsies and 29/79 (36.71%) small surgical biopsies were unsatisfactory. 16/25 (64%) cases ordered on cell block alone and 8/25 (32%) with DQ smears were unsatisfactory (p=0.024). The unsatisfactory rates for small surgical biopsies (36.71%) and cytologic specimens with DQ smears (32%) were not significantly different (p=0.671).
Conclusions: While large surgical biopsies yield the lowest rate of unsatisfactory samples (6.94%) as might be expected, the utilization of DQ stained smears for molecular testing has improved the adequacy of cytologic samples, providing a minimally invasive alternative to surgical biopsy when molecular analysis of tumor material is necessary. We recommend the use of DQ stained smears for molecular analysis when cell block material is inadequate.

318 Cytological Diagnosis of Papillary Thyroid Carcinoma With Tall Cells: A Comparative Analyses of ThinPrep and Conventional Smear Cytology

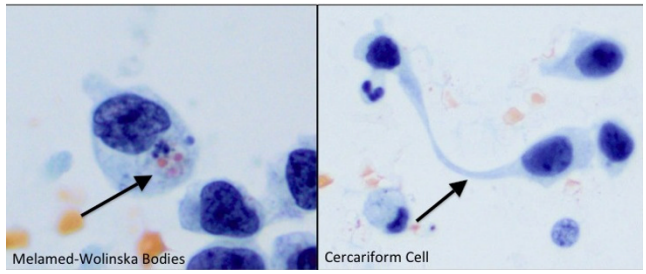
Jordan Arkin, Theresa Scognamiglio, Rana Hoda, Rema Rao. New York Presbyterian Hospital – Weill Cornell Medical Center, New York, NY.
Background: Tall cell variant of papillary thyroid carcinoma (TCV-PTC) and papillary thyroid carcinoma with tall cell features (PTC-TCF) are relatively aggressive tumors when compared to classic PTC (c-PTC). They show increased incidence of extrathyroidal extension and metastasis, thus requiring more aggressive treatment options. Pre-operative diagnoses of these entities using fine needle aspiration cytology (FNAC) may help triage appropriate management in these cases. However, diagnosing TCV-PTC and PTC-TCF in FNAC is quite challenging. With ThinPrep (TP) being increasingly used in FNAC we aimed to investigate the cytologic features of TCV-PTC and PTC-TCF on TP. Cytologic features of these tumors have been previously described on conventional smear preparations (CS), but the literature on their appearance on TP has been scant. Herein, we compare the cytology of TCV-PTC and PTC-TCF on TP in comparison to CS.
Design: Cases of TCV-PTC and PTC-TCF that had both FNAC (CS and TP) and surgical follow up (F/U) from our institution, over a 7-year-4 month period (03/2007 to 07/2014), were retrospectively reviewed. Appropriate controls were used.
Results: All of the 16 study cases (7 TCV-PTC and 9 PTC-TCF) and the 9 control cases (4 c-PTC and 5 follicular variant PTC) had CS, TP, and F/U during the study period. On review, both TCV-PTC and PTC-TCF combined (PTC-TC) and controls showed syncytial and monolayered groups on both TP and CS. Features favoring PTC-TC on TP and CS, respectively, were as follows: TC in groups (94% and 75%), cells with oncocytic cytoplasm (75% and 69%), and distinct cell borders (50% and 62%). Features of PTC-TC prominent on TP over CS were as follows: single TC (63% vs 0%), TC with cytoplasmic tails (75% vs 56%), and the newly identified feature of cytoplasmic cuff (cytoplasmic rim) encircling syncytia and monolayered groups (38% vs 25%). The only feature of PTC-TC more prominent on CS over TP was the presence of intranuclear soap-bubble pseudoinclusions (69% vs 25%).
Conclusions: PTC-TC including TCV-PTC and PTC-TCF can be cytologically detected on TP cytology. Prominent features seen on TP cytology include architectural features such as TC in groups and single TC as well as cytological features such as TC with cytoplasmic tails, cytoplasmic cuffs surrounding syncytia and monolayered groups, cells with oncocytic cytoplasm, and distinct cell borders.

319 Evaluation of the Afirma Gene Expression Classifier in Cytologically Indeterminate Thyroid Nodules: A Pilot Study of 45 Fine Needle Aspiration Cases

Jordan Arkin, Theresa Scognamiglio, Rana Hoda, Tamar Giorgadze. New York Presbyterian Hospital – Weill Cornell Medical Center, New York, NY.
Background: Fine needle aspiration (FNA) has become standard of care for the initial evaluation of thyroid nodules. However, 15-30% of FNA results are indeterminate, the majority of which are ultimately benign on surgical follow up (F/U). The Afirma gene expression classifier (GEC) has increasingly been used to help further characterize indeterminate FNA and guide surgical management. We investigated the performance of the GEC in the clinical setting of a large academic medical center.
Design: 45 indeterminate FNAs were identified in 17 months (03/'13-08/'14) for which GEC was performed. Indeterminate FNA diagnoses included Atypia of undetermined significance/Follicular lesion of undetermined significance (AUS/FLUS) and Follicular neoplasm/Suspicious for follicular neoplasm (FN/SFN). Specimens were collected and sent for GEC analysis at the time of FNA according to the manufacturer's protocol and at the discretion of the treating physician.
Results: All 45 FNAs were adequate for GEC. Cytological diagnoses were: 35 AUS/FLUS and 10 FN/SFN. Afirma results were benign in 16 (36%, 14 AUS/FLUS, 2 FN/SFN) and suspicious in 29 (64%, 21 AUS/FLUS, 8 FN/SFN). Surgical F/U was available for 24/29 (83%) suspicious cases (16 AUS/FLUS, 8 FN/SFN). Of these, 9/24 (38%) were malignant, while 15/24 (62%) were benign on surgical F/U. The 9 malignant cases included 7 follicular variant papillary thyroid carcinoma (PTC), 1 solid variant PTC, and 1 follicular carcinoma (FC). The cytological diagnoses on these malignant cases were: 5 AUS/FLUS and 4 FN/SFN. The difference in malignancy rates for AUS/FLUS and FN/SFN (31% & 50%, respectively) was not statistically significant (*p value 0.32*). Of the 45 FNAs, 5 were oncocytic lesions (OL) (4 AUS/FLUS, 1FN). 4 of 5 (3 AUS/FLUS, 1 FN) were classified as suspicious (80%). Surgical F/U in 3 suspicious OL revealed malignancy (FC) in 1 case (33%). Clinical F/U was elected in lieu of surgery in all 16 cases with benign GEC results.
Conclusions: The Afirma GEC is a useful adjunct for management of indeterminate thyroid nodules. The rate of malignancy (38%) in nodules suspicious by Afirma GEC in our study is similar to that reported in the literature. The GEC impacted clinical care with the majority of patients with suspicious GEC undergoing surgery. Our results with the Afirma GEC suggest it may be less reliable in OL, although the limited number of OL cases in our study makes it difficult to make any significant conclusion.

320 Metastatic Urothelial Carcinoma on Fine Needle Aspiration: Review of Clinical, Cyto-Histological and Immunocytochemical Data

Saad Baqai, Michael Chaump, Tamar Giorgadze, Rana Hoda. Weill Cornell Medical College, New York, NY; Danbury Hospital, Danbury, CT.
Background: Distinguishing metastatic urothelial carcinoma (Met-UC) from other epithelial malignancies on fine needle aspiration (FNA) can be a diagnostic challenge. Yet, the literature on cytological features of Met-UC on FNA is scant (*Kaur. Journal of Cytology, 2012; 29:2*). Herein, we review existing and a newer cytological criterion of Melamed-Wolinska Bodies (MWB) in FNA of Met-UC. MWB are round eosinophilic cytoplasmic inclusions seen in urinary cytology and indicate degenerative changes. This feature, to our knowledge, has thus far, not been reported in Met-UC.
Design: Patients with Met-UC were identified during a 57-month-period (01/2010-09/2014). All had histologically-proven primary UC. Cytomorphological features were assessed on Diff-Quik and Pap-stained direct smears and H&E-stained cell blocks. Metastatic carcinomas (Met-Ca) of other primary sites were reviewed as controls. Clinical, immunocytochemical and histopathological data was also reviewed.
Results: There were 21 FNAs with Met-UC from 20 patients (13 men and 7 women; age range, 61-87 years; mean 69). Metastatic sites were: 7, lymph nodes; 4, liver, 4, lung and 6, soft tissue and bone. Cytomorphological features assessed in the 21 cases included: Architectural Features of single cells, 18 (86%); papillary fragments, 8 (38%) and syncytia, 8 (38%); Cytological Features of cercariform cells, 15 (71%), cell cannibalism 16 (76%), MWB, 9 (43%) and squamous differentiation, 2 (9.5%). Immunocytochemical stains for CK7, p63 and CK903 were positive in 100%, 94% and 100% cases of Met-UC respectively. The 10 control cases were Met-Ca of lung, colon, breast, gynecologic and prostate origin. Frequency of these features in cases assessed were: single cells, 9 (90%); papillary fragments, 1 (10%); syncytia, 1 (10%); cercariform cells, 2 (20%); cell cannibalism, 2 (20%); and MWB, 1 (10%).



Conclusions: Cytomorphological features that may distinguish Met-UC from other Met-Ca include presence of cercariform cells, cell cannibalism, papillary fragments and the newer criterion of MWB. Review of pathology from primary UC and immunocytochemistry would be valuable.

321 Can the Individual Pathologists' Urothelial Atypia To High Grade Urothelial Carcinoma Ratio Be Used as a Quality Assurance Indicator?

Guliz Barkan, Grazina Chatt, Eva Wojcik, Stefan E Pambuccian. Loyola University, Maywood, IL.
Background: The reported percentage of "atypical urothelial cells" (AUC) diagnosis ranges between 2-23% in different institutions. For the most part the reason for such a wide variation is because of a lack of well-established criteria for atypia for urinary tract cytology (UTC). The Paris System for reporting UTC, an international effort to standardize reporting of UTC, will not only establish the necessary criteria for the diagnostic categories but also is predicted to reduce the atypia rate in UTC.The aim of this study was to evaluate the possibility of using quality control indicators in UTC akin to that of ASCUS:LSIL ratio of gynecologic cytopathology, to monitor the equivocation of individual pathologists, and potentially reduce the rate of equivocal diagnoses.
Design: All UTC diagnoses made by individual pathologists from 1/1/2000 to 9/18/2014 were identified through a search of our institution's electronic medical records. The percentages of "Negative for malignancy" (NEG), AUC, suspicious for high grade urothelial carcinoma (SHGUC), and high grade urothelial carcinoma (HGUC) were calculated for individual pathologists and compared to the laboratory mean. Similarly, AUC/HGUC, (AUC+SHUC)/HGUC and (AUC+SHUC)/(HGUC+NEG) ratios were calculated for each pathologist and for the institution.
Results: Excluding specimens considered unsatisfactory, a total of 31,284 UTC cases were diagnosed at our institution during the 14-year span of the study. 27,966 (89.4%) were diagnosed as NEG, 1,905 (6.1%) as AUC, 392 (1.25%) as SHGUC, and 1,021 (3.3%) as HGUC. Figure shows the percentages of individual pathologists and the institution as a whole.

	NEG	AUC	SHGUC	AUC+SUSP	HGUC	AUC:HGUC	(AUC+SHGUC)/ HGUC	(AUC+SHGUC)/ (HGUC+NEG)	TOTAL
Pathologist 1	71.46%	11.10%	1.47%	12.57%	3.40%	3.27	3.7	0.17	883
Pathologist 2	87.00%	4.61%	0.67%	5.27%	2.45%	1.88	2.15	0.06	5101
Pathologist 3	86.66%	4.25%	1.27%	5.51%	2.31%	1.84	2.39	0.06	1342
Pathologist 4	58.10%	15.64%	2.79%	18.44%	5.03%	3.11	3.67	0.29	179
Pathologist 5	86.60%	4.56%	0.80%	5.36%	2.68%	1.7	2	0.06	373
Pathologist 6	85.71%	4.58%	0.98%	5.57%	3.15%	1.45	1.77	0.06	5585
Pathologist 7	76.75%	8.93%	1.46%	10.39%	2.47%	3.61	4.2	0.13	1983
Pathologist 8	84.73%	4.89%	1.22%	6.11%	3.05%	1.6	2	0.07	3045
Pathologist 9	84.73%	5.34%	1.22%	6.56%	2.14%	2.5	3.07	0.08	655
Pathologist 10	83.44%	5.84%	1.06%	6.90%	2.76%	2.11	2.5	0.08	4812
Pathologist 11	59.92%	16.54%	2.72%	19.26%	1.56%	10.63	12.38	0.31	514
Pathologist 12	82.78%	5.35%	1.38%	6.73%	3.77%	1.42	1.79	0.08	9109
Department Total	27966	1905	392	2297	1021	1.87	2.25	0.08	31284
%	**89.39%**	**6.09%**	**1.25%**	**7.34%**	**3.26%**				100.00%

Conclusions: Individual pathologists had a wide range of %AUC diagnoses (4.3-16.5%), AUC:HGUC ratios (1.4-10.6, institution 1.9), (AUC+SGUC)/HGUC ratios (1.8-12.4, institution 2.3) and (AUC+SGUC)/(NEG+HGUC) ratio (0.06-0.31, institution 0.08). We propose using these measures to monitor individual pathologists' use of "equivocal" UTC diagnoses and consider intervention for significant deviations from the institution's mean.

322 In Preparation for the Paris System for Reporting Urinary Tract Cytopathology (TPSRUTC): Observations From the 2014 Supplemental Questionnaire of the College of American Pathologists (CAP) Cytopathology Interlaboratory Comparison Program (CICP)

Guliz Barkan, Z Laura Tabatabai, Charles Sturgis, Daniel Kurtycz, Rhona Souers, Ritu Nayar. Loyola University, Maywood, IL; University of California, San Francisco, CA; Cleveland Clinic, Cleveland, OH; University of Wisconsin, Madison, WI; CAP, Northfield, IL; Northwestern University, Chicago, IL.

Background: Information about TPSRUTC has been disseminated since its inception in 2013; however, the daily practice of UTC are not well known. This study aims to assess UTC practice patterns across a variety of pathology laboratories in order to aid in the shaping and implementation of TPSRUTC.

Design: A questionnaire was designed to gather information about UTC practices and mailed in July 2014 to 2,116 laboratories participating in the CAP-CICP. The participating laboratories' answers were collected and summarized.

Results: Of 879/2116 (41%) laboratories that participated, 745/879 (84.8%) reported processing UT specimens (UTS) in-house. Rates of processing voided urine (VU), bladder washing/barbotage (BWB), and catheterized urine (CU) specimens were 735/738 (99.6%), 639/738 (86.6%), 653/738 (88.5%) respectively. The most commonly used preparation technique for UTS was ThinPrep (57.4%) and Cytospin (45.5%). 88/197 (44.7%) of the laboratories reported preparing a cell-block (CB) in <10% of the cases. 295/707 (41.7%) and 244/707 (35.7%) laboratories reported using "adequacy criteria" for VU and BWB, respectively. Over 95% of the laboratories reported to use the general categories: Negative, Atypical, Suspicious, and Positive. For the Atypical category, 617/705 (87.5%) reported to use specific terminology "atypical urothelial cells present". The finding of polyomavirus was classified as "negative" in 63.6% of laboratories and "atypical" in 29.8%. 18.1% of laboratories performed ancillary testing (83.6% performed UroVysion). 251/699 (35.9%) of the laboratories reported awareness of TPSRUTC.

Conclusions: Majority of laboratories report using "adequacy criteria" in UTS assessment, suggesting a need for defining and establishing such criteria. Over 85% of the laboratories report processing BWB and CU specimens. Current use of similar reporting categories suggests that transition to TPSRUTC, at least in the US, may be relatively smooth. However, since only one third of laboratories are aware of the proposed system, additional educational efforts regarding TBSRUTC are warranted.

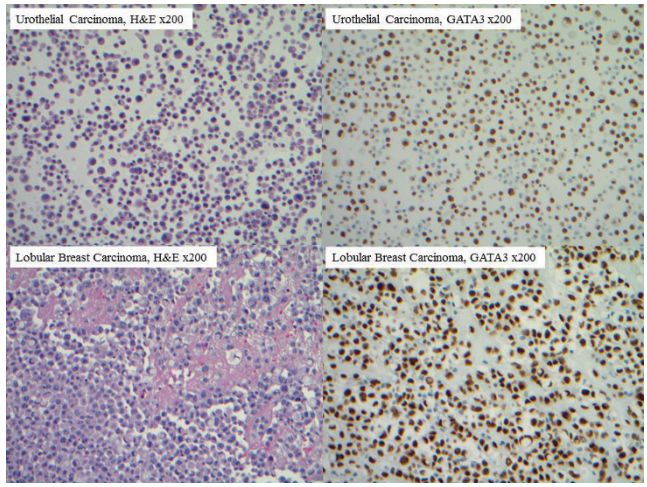
323 GATA3 Expression Is Useful in Identifying Metastatic Carcinomas of Breast and Urinary Bladder Origin

Jane Bernstein, Angelique Levi, David Chhieng, Diane Kowalski. Yale University School of Medicine, New Haven, CT.

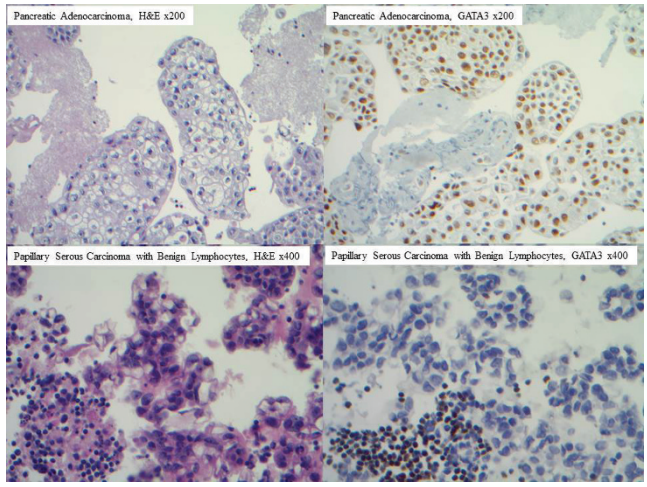
Background: GATA-binding protein 3 (GATA3) is a transcription factor that has been well documented as a moderately sensitive marker for breast and bladder epithelium in surgical pathology. On the contrary, little has been published about the utility of GATA3 immunostaining in cytology specimens. The aim of this study was to evaluate the role of GATA3 in malignant cytology specimens from various sites with emphasis on its use in identifying metastatic mammary and urothelial carcinomas, two lesions which often pose morphologic and immunohistochemical diagnostic challenges.

Design: Cell block sections from 48 fine needle aspirates and body fluid cytology specimens were stained with an anti-GATA3 monoclonal antibody. Specimens included confirmed metastatic carcinomas of the breast, urothelium, female genital tract, gastrointestinal tract, pancreas, lung, and epithelioid angiosarcoma. Five cases of reactive mesothelial cells were stained along with one reactive broncheoalveolar lavage specimen.

Results: Nuclear staining for GATA3 was found to be present in 100% of metastatic breast carcinoma specimens (20 cases) and 100% of metastatic urothelial carcinoma specimens (7 cases).



A case of metastatic pancreatic adenocarcinoma, a case of lung squamous cell carcinoma, and the epithelioid angiosarcoma case also exhibited GATA3 staining. In addition, 11 cases demonstrated nuclear positivity in background non-neoplastic lymphocytes.



Conclusions: Our results show that GATA3 is a useful marker to be included in a panel for the differential diagnosis of metastatic breast or urothelial carcinomas, given its high rate of sensitivity for these neoplasms. The staining of non-neoplastic lymphocytes can serve as a built-in positive control, which is especially useful in cytologic material. The utility of GATA3, however, is limited by its specificity, shown to be 86% in this study. It is important to be aware of the occasional GATA3 positivity in malignancies of other primary sites.

324 SATB2 and Cadherin-17 in Cytology Specimens: Do These New Immunostains Help in Differentiating Metastatic Colorectal Adenocarcinoma From Adenocarcinomas of Other Origins?

Tamar Brandler, Fatima Zahra Jelloul, Kasturi Das, Daniel Soto, Lisa Rosen, Tawfiqul Bhuiya. Hofstra North Shore Long Island Jewish School of Medicine, Lake Success, NY.

Background: SATB2 and Cadherin-17 immunoexpression has recently been described in surgical pathology literature to have value in establishing colorectal origin of metastatic adenocarcinoma. However, literature on SATB2 and Cadherin-17 staining in metastatic colorectal adenocarcinoma (MCA) in cytology is limited. Here we evaluate the utility of SATB2 and Cadherin-17 in the diagnosis of MCA in cytology specimens and compare these two novel markers with the standard gastrointestinal immunohistochemistry panel in an attempt to formulate the most practical panel for limited available material.

Design: A search was conducted in our information system for cytology cases of MCA between 1/1/01 and 12/31/13, which generated 124 potential cases. 58 had cell blocks with tissue and were retrieved. 21 MCA cytology cases and 37 metastatic non-colorectal adenocarcinomas (non-MCA) (including breast, endometrial, lung, ovarian, pancreatic, prostatic) were stained for SATB2, Cadherin-17, and the standard panel of CK7, CK20 and CDX2. Staining intensity (weak, moderate, and strong) and percentage of positive cells were recorded. >1% of nuclear SATB2 and membranous Cadherin-17 staining was considered as positive.

Results: MCA cytology cases stained positive for SATB2 and Cadherin-17 in 47.62% (10/21) and 100% (21/21) of cases respectively, with varying staining intensity for SATB2 and mainly strong staining intensity for Cadherin-17. The sensitivity and specificity for SATB2, Cadherin-17 and the standard panel of immunostains are reported in table 1.

Immunostain(s)	Sensitivity (95% CI) (%)	Specificity (95% CI) (%)
SATB2	47.62 (25.71, 70.22)	91.89 (78.09, 98.30)
Cadherin-17	100.00 (83.16, 100.00)	86.21 (68.34, 96.11)
SATB2 + Cadherin-17	100.00 (83.16, 100.00)	79.31 (60.28, 92.01)
Standard Panel (CK7, CK20, CDX2)	76.19 (52.83, 91.78)	97.22 (85.47, 99.93)

Conclusions: Our study reinforces that the standard gastrointestinal immunohistochemistry panel remains the gold standard for distinguishing MCA from non-MCA. Cadherin-17 is best used as an adjunct to the standard panel to differentiate MCA from Non-MCA due to its high sensitivity and specificity. The role of SATB2 however, is limited due to its low sensitivity in cytology specimens with minimal material.

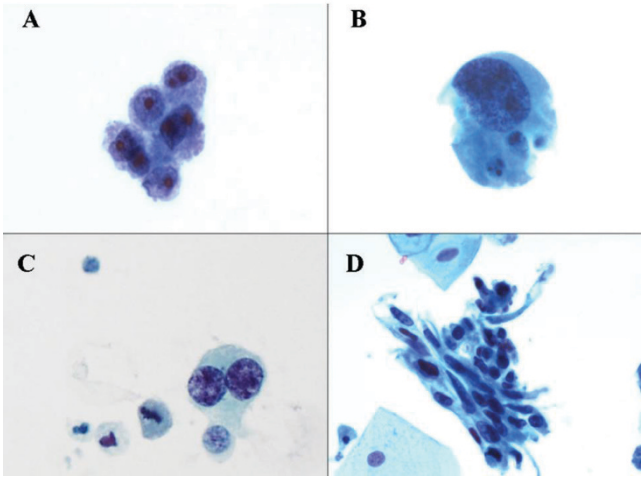
325 Non Urothelial Malignancy in Urine Cytology: Frequency, Detection, and Cytological Features

Fadi Brimo, Ayoub Nahal, Yonca Kanber, Derin Caglar, Manon Auger. McGill University Health Center, Montreal, QC, Canada.

Background: We aimed to evaluate the true incidence and types of non-urothelial malignancy detected in urine cytology in a large cytology laboratory.

Design: All urine cytology cases reported to be positive or suspicious for non-urothelial carcinoma (CA) between 2008-2014 were retrieved. In addition all cases reported as positive or suspicious for urothelial CA in which the follow-up biopsy showed malignancy of non-urothelial origins were included.

Results: 1515 positive urine cytology specimens were included. Of those 1075 had a follow-up biopsy. Only eight cases represented non-urothelial malignancy on cytology. The proper clinical history was available in only three cases at time of sign-out. The final diagnosis was as follows: prostatic adenocarcinoma (n=5), colorectal adenocarcinoma (n=2), melanoma (n=1). Two cases were originally reported as suspicious for urothelial CA, two as positive for urothelial CA, two as suspicious for non-small cell CA, and two as non-small cell CA. On review significant overlapping features with urothelial CA (high N:C ratio, eccentric nuclei, coarse chromatin, irregular nuclear membrane) were evident in five cases. In the rest, the differentiating cytological features that could have suggested a non-urothelial origin were: 1- foamy cytoplasm in adenocarcinoma; 2-monotonous round nuclei with prominent cherry-red nucleoli in prostatic adenocarcinoma; 3- columnar nuclei in colorectal adenocarcinoma; 4-frequent bi/ multinucleation in melanoma.



Conclusions: Non-urothelial malignancy comprises only 0.007% of the 'positive' urine cytology cases. The two most common diagnoses are prostatic and colorectal adenocarcinoma. Significant cytological overlap with urothelial CA may be present even on review and with the appropriate clinical history. In other instances, the lack of the typical features of urothelial CA combined with the cellular degeneration and/or the lack of relevant clinical history result in a tendency to assign a'suspicious' rather than a 'positive' diagnosis.

Figure 1. A- prostatic Ca with typical cytological features, B- prostatic adenocarcinoma mimicking urothelial CA, C- melanoma mimicking urothelial CA but with frequent binucleation, D- colorectal adenocarcinoma with typical cytological features.

326 Impact of Flow Cytometry in Renal Cytopathology

Jennifer Bynum, Amy Duffield, Syed Ali. Johns Hopkins, Baltimore, MD.

Background: Fine needle aspiration (FNA) is commonly used as a diagnostic tool for the evaluation of lymphoproliferative diseases in many organs, including the kidney. Cytomorphology alone is often insufficient for the diagnosis and subclassification of lymphomas, so flow cytometry (FC) may be used as an ancillary study to provide a more detailed characterization of these neoplasms.

Design: Renal FNAs performed at our institution from January 1993 – August 2014 were reviewed for FC data or a diagnosis of lymphoma without FC data. Specimens were obtained by ultrasound-guidance with onsite evaluation of adequacy, smears were stained with Diff-Quik and Papanicolaou stains, and needle rinses saved in Hanks balanced salt solution were submitted for FC analysis.

Results: A total of 586 renal FNAs were collected over the study period. Thirty cases (5.1%) had FC analysis and 11 (1.9%) cases had a diagnosis of lymphoma without FC data. Lymphoma was diagnosed 35 times on cytopathology specimens. Cytomorphology alone was diagnostic of lymphoma in 15 cases, and both cytomorphology and FC were consistent with a diagnosis of lymphoma in 20 cases. One largely necrotic specimen that was also negative on FC showed large B-cell lymphoma on subsequent biopsy. Subsequent biopsy or surgical resection confirmed the lymphoma diagnoses in two cases. In 26 cases, the patient had a biopsy diagnostic of lymphoma taken from another site either before or after the cytology specimen. One case with cytomorphology concerning for lymphoma had negative FC, but a paraaortic lymph node biopsy two weeks later showed diffuse large B cell lymphoma. In the remaining 5 cases, the diagnoses and subsequent treatment rested on the cytologic and flow cytometric evaluation. In all 5 cases, a lesion that was morphologically concerning for lymphoma on the aspirate showed no phenotypic abnormality on FC. In 4 of these cases, FC provided information that suggested that the neoplasm was non-hematopoietic, and the final case was Hodgkin lymphoma, which is not a diagnosis routinely made on flow cytometry.

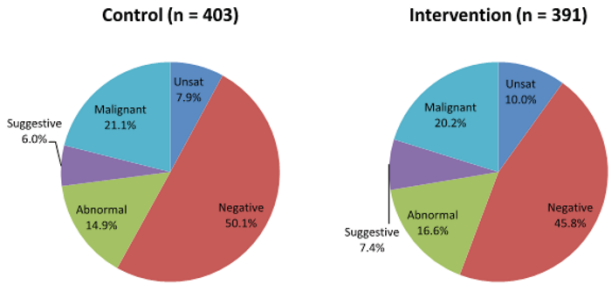
Conclusions: FC is a useful adjuvant diagnostic test for renal FNAs performed on patients with suspected lymphoproliferative disorders. It is particularly useful for subclassification of lymphomas and for confirming the diagnosis when there is insufficient material for immunohistochemistry. FC should be interpreted with caution when a sample is limited or when there is suspicion of Hodgkin lymphoma, and further work-up is warranted when cytomorphology is concerning for lymhoma but FC is negative.

327 Impact of Routine Cell Block Preparation on Results and Turnaround Time for Head and Neck Fine Needle Aspirates

Michael Carter, David Moore, Rebecca MacIntosh, Martin Bullock. Dalhousie University, Halifax, NS, Canada.

Background: Fine needle aspiration (FNA) of head and neck masses is a commonly-used technique for providing cytology specimens to guide patient management. Tissue cell blocks are sometimes made from these specimens to provide an additional diagnostic tool. Our institution implemented a program wherein cell blocks were routinely produced for all non-parotid gland head and neck FNAs. This was a departure from the earlier policy of only producing cell blocks in cases where one was requested by the pathologist. The program was evaluated in terms of its impact on diagnosis and specimen turnaround time (TAT).

Design: A retrospective study was carried out using electronic pathology records at our institution. The intervention group consisted of all non-parotid gland head and neck FNAs obtained in the 15-month period following implementation of a program of routine cell block preparation (n = 391). The control group consisted of the same specimens obtained in the 15 months prior to implementation of this program (n = 403). The groups were compared with regards to diagnostic distribution into five categories - Unsatisfactory, Benign, Abnormal, Suggestive of malignancy, and Malignant. TAT from specimen collection to issuance of a pathology report was also compared for the two groups.



Results: There was no significant difference in diagnostic distribution between the two groups (p=0.59, chi square test). The lack of a difference in the frequency of Abnormal diagnoses (16.6% in the intervention group, 14.9% control group) suggests that the level of diagnostic certainty was not improved by using cell blocks for all cases. Similarly, the unchanged frequency of Unsatisfactory diagnoses (10.0% intervention, 7.9% control) indicates that the program did not reduce this undesirable metric. TAT was also unchanged between the groups (mean 3.83 days in the intervention group vs. 3.95 days control group, median = 3 days for both groups, p=0.74 by chi square test). The cost of the program was estimated at CAD$53.60/cell block, or CAD$16,771/year. Cytological-histological correlation is underway.

Conclusions: Implementation of routine cell blocks for head and neck FNAs failed to improve diagnostic certainty or specimen turnaround time despite incurring substantial cost.

328 Predicting the Histologic Subtype of Lung Adenocarcinomas Using Cytologic Specimens

Nina Chang, Jordan Sim, Marcio Gomes, Harman Sekhon. University of Ottawa, Ottawa, ON, Canada.

Background: The 2011 IASLC/ATS/ERS classification of lung adenocarcinoma (ADC) have identified histologic grades that correlate with tumour behaviour and prognosis: low (non-mucinous lepidic), intermediate (acinar and papillary) and high (micropapillary and solid). While subtyping has been traditionally made on histological specimens, cytologic samples from fine needle aspiration biopsies are becoming more frequent and recognition of patterns on cytology may assist management by selecting early stage low grade ADCs for follow-up rather than resection, distinguishing synchronous

primaries from intrapulmonary metastasis, and prioritizing high grade ADCs for resection in cases of synchronous tumours. In non-resectable cases, subtyping may provide prognostic information and identify features associated with certain molecular profiles. Here we analyze criteria for determining histologic subtypes of lung ADC on cytologic specimens.

Design: Lung ADCs from 2012-2014 with cytologic and resection specimens were reviewed. Cases with non-diagnostic cytology were excluded, as were mucinous ADCs and ADCs with pleomorphic and neuroendocrine components. Cytologic smears (CS) and cell blocks (CB) from 80 consecutive cases were reviewed by a pathologist and two trainees who were blinded to final histologic subtype to identify useful histologic criteria. These criteria were correlated with the predominant subtype in resection specimens via Fisher's exact test using SPSS v20.

Results: Solid predominant (SP) ADC significantly correlated with necrosis, single atypical cells (SAC), nuclear atypia, nuclear crowding, cytoplasmic hyaline globules and cytoplasmic inflammatory cells on CS or CB. SP ADC correlated with significant nuclear atypia on CB ($p<0.001$) with 88% sensitivity (Sn) and 72% specificity (Sp). SP ADC correlated with large sheets of cells on CB ($p<0.001$) with 71% Sn and 89% Sp. Micropapillae on CB correlated with micropapillary pattern ($p=0.001$) with 71% Sn and 89% Sp. Papillary predominant (PP) ADC significantly correlated with SAC, strips of cells, apical caps and papillae on CB. No criteria predicted acinar predominant ADC. Other features, such as hemosiderin-laden macrophages, nuclear inclusions and nuclear grooves, were not useful.

Conclusions: We report for the first time cytologic features associated with solid and papillary patterns of ADC. While the sensitivity of some criteria was low, use of these criteria in combination may improve sensitivity. If validated, these criteria will impact treatment decision-making and provide additional prognostic information.

329 Estimating the Risk of Abnormal Cervical Papanicolaou Test (PAP) in Female Renal Transplant Recipients (RTR) as Compared With Age-Matched Controls

Shelley Chang, Matthew Tarin, Po Fung, Amer Karam, Jianyu Rao. University of California, Los Angeles, CA; Stanford University Medical Center, CA.

Background: It has been perceived that female renal transplant recipients (RTR) are at increased risk for HPV-related cervical precancerous lesions, however the exact risk for such patient population relative to general population, especially the risk of abnormal cervical Papanicolaou (PAP) cytology, is unknown. Accurate estimation of the risk among RTR may help to develop specific guidelines to screen cervical cancer in these women.

Design: We conduct an age matched-cohort study between Jan 2000 to Dec 2011 at an academic center. The risk of having an abnormal cervical PAP test was compared between female RTR and age-matched controls undergoing cervical PAP test on the same day. Transplantation records, cervical PAP cytology and tissue diagnosis, demographics, and relevant risk factors, including age, gender, number of previous transplants, native kidney pathology, and previous PAP cytology results were obtained through electronic medical records. Odds ratios were calculated using logistic regression.

Results: A total of 1283 female patients underwent kidney transplantation during the study period, 91 (7%) of whom received at least one cervical PAP test at our institution subsequent to kidney transplantation. Renal transplant recpient women were significantly more likely to have at least ASCUS (OR 6.91 [95% CI: 3.3-14.7]), at least LSIL (OR 6.91 [95% CI: 3.3-14.7]), or at least ASC-H (OR 10.5 [95% CI: 1.2-91.5]) on index cervical PAP cytology test as compared with age-matched controls. Nine (5%) of the controls and 23 (25%) of the renal transplant recipients obtained further tissue diagnosis. Among those with tissue diagnoses, there is a trend towards increased frequency of least CIN1/koilocytic change (78% RTR vs 56% controls, P=0.22) and at least CINII (35% RTR vs 0 % controls, P=0.07) in renal transplant recipients as compared with controls.

Conclusions: Renal transplat recipients are at nearly 7-fold increased risk for having an abnormal PAP exam subsequent to transplantation as compared with age-matched controls. Increased surveillance using PAP tests may be useful for cancer prevention among renal transplant recipients.

330 Differentiating Non-Infiltrative, Non-Invasive Follicular Variant of Papillary Thyroid Carcinoma From Classical Type Papillary Thyroid Carcinoma on Fine Needle Aspiration

Sue Chang, Markus Eszlinger, Ralf Paschke, Jeffrey Krane, Justine Barletta. Brigham & Women's Hospital, Boston, MA; Universität Leipzig, Leipzig, Germany.

Background: If conservative management (i.e. lobectomy only) were advised for non-infiltrative, non-invasive follicular variant of papillary thyroid carcinoma (FVPTC) on the basis of their extraordinarily low malignant potential, it would be important to distinguish them from classical type PTC on fine needle aspiration (FNA). The aim of this study was to determine if non-infiltrative, non-invasive FVPTCs can be distinguished from classical type PTC by cytologic evaluation and molecular analysis of preceding FNA specimens.

Design: We identified a cohort of 40 non-infiltrative, non-invasive FVPTCs and classical type PTCs that according to The Bethesda System for Reporting Thyroid Cytopathology were diagnosed as suspicious for malignancy (SUS) or malignant and had undergone molecular analysis for *BRAF, RAS, PAX8*/*PPARγ,* and *RET/PTC* alterations. ThinPrep cytology slides were reviewed for the presence of the following features: predominance of microfollicles, predominance of small tumor sheets, papillae, and nuclear pseudoinclusions or psammomatous calcifications.

Results: Our cohort included 12 (30%) non-infiltrative, non-invasive FVPTCs and 28 (70%) classical PTCs. Non-infiltrative, non-invasive FVPTCs were significantly more frequently diagnosed as SUS on FNA and showed a predominance of microfollicles, while classical PTCs were significantly more frequently diagnosed as malignant and associated with small tumor sheets, papillae, and pseudoinclusions or psammomatous calcifications. Of the FVPTCs with successful molecular analysis, 3 (30%) had a *RAS* mutation, 1 (10%) had a *PAX8*/*PPARγ* rearrangement, and 5 (50%) were wild-type (WT), whereas for the classical PTCs with successful molecular analysis, 14 (61%) harbored a *BRAF* V600E mutation, 1 (4%) had an *NRAS* mutation, and 8 (35%) were WT.

	Classical (%), n=28	FVPTC (%), n=12	P value
Suspicious on FNA	6 (21)	11 (92)	
Malignant on FNA	22 (79)	1 (8)	
Microfollicle predominant	1 (4)	7 (58)	0.0003
Small sheet predominant	27 (96)	4 (33)	
Papillae	14 (50)	0	0.0027
Pseudoinclusions	23 (82)	0	
BRAF*	14 (61)	0	0.0014
*Molecular analysis was successful for 23 classical and 10 FVPTCs.			

Conclusions: Our findings suggest that non-infiltrative, non-invasive FVPTC can be differentiated from classical type PTC prior to surgery on the basis of cytologic features and molecular analysis of the preceding FNAs.

331 Fine Needle Aspiration of Lymphoma Involving the Pancreas: A 16-Year Retrospective Study of 25 Cases From a Single Institution

Shaoxiong Chen, Howard Wu, Xiaoyan Wang, Jingmei Lin, Shanxiang Zhang, Jiehao Zhou, Harvey Cramer. Indiana University, Indianapolis, IN.

Background: Lymphoma involving the pancreas is rare and commonly associated with lymph node involvement in other locations. Patients with either pancreatic lymphoma or adenocarcinoma may present with similar clinical findings. It is crucial to correctly distinguish between these two entities since their treatments are so vastly different. In this study, we evaluate our experience diagnosing pancreatic lymphoma by FNA.

Design: A computerized search of our LIS was performed for the 16-year period from 1997 through 2013 to identify all cytology and surgical pathology cases in which the diagnosis of lymphoma involving the pancreas was rendered. All cytology, surgical pathology reports and related clinical histories were retrospectively reviewed. Slides from the cytology and surgical cases were re-examined.

Results: A total of 25 FNA cases were collected over a 16-year period. The age of the patients ranged from 44 to 89 years with a mean age of 67. The male to female ratio was 1.3. The size of the pancreatic lesions ranged from 2.0 to 20.0 cm. Five cases had a prior history of lymphoma. Among 20 newly diagnosed cases of pancreatic lymphoma, 12 were associated with lymph node involvement in the peri-pancreatic region or other locations and in 8 cases the possibility of other sites of involvement could not be completely excluded due to inadequate documentation in the patients' electronic medical records. Fourteen cases had concurrent flow cytometry that was utilized during evaluation of the FNA smears. The FNA diagnoses were classified as follows: positive (17 cases, 68%), atypical/suspicious (5 cases, 20%), lymphoma vs carcinoma (1 case, 4%), negative (1 case, 4%), and nondiagnostic (1 case, 4%). The 17 positive cases included 11 large B-cell lymphomas, 3 follicular lymphomas (FL), 1 B-cell lymphoma NOS, 1 anaplastic large cell lymphoma and 1 Hodgkin lymphoma. Histological follow-up was obtained in only 4 of the 17 positive FNA cases and showed 1 FL and 3 diffuse large B-cell lymphomas (DLBCL). Follow-up of 3 of 5 atypical/suspicious cases showed 1 FL and 2 DLBCL. Histological follow-up of FNA cases diagnosed as negative (1) and nondiagnostic (1) revealed FL and DLBCL, respectively. The case of lymphoma vs carcinoma turned out to be DLBCL on histological follow-up.

Conclusions: Lymphoma involving in the pancreas can be reliably diagnosed by FNA. In our study, the false negative and nondiagnostic rates were each 4%. In most cases (13/17), confirmatory histopathologic evaluation was not obtained and patients were treated solely on the basis of the FNA diagnosis.

332 Performance of Cobas HPV Test in Atypical Squamous Cells of Undetermined Significance (ASC-US) and Atypical Glandular Cells (AGC) Categories

Crystal Cordell, Roxanne Mody, Haijun (Steve) Zhou, Steven Shen, Eric Luna, Donna Armylagos, Mary Schwartz, Dina Mody, Yimin Ge. Houston Methodist Hospital, Weill Medical College of Cornell University, Houston, TX; University of Texas, Health Science Center, Houston, TX; BioReference Laboratories, Houston, TX.

Background: Roche Cobas HPV test was FDA approved for co-testing women >30 years and ASC-US triage in 2011 and as a primary screen in 2014. This study aims to evaluate the presence of HPV 16/18 and non-16/18 genotypes in women with ASC-US and AGC using the Cobas platform and to correlate with follow-up biopsy.

Design: The cytology-histology-HPV correlation database from March 1, 2013 to June 30, 2014 from BioReference Laboratories in Houston, Texas was examined. C^2 test was performed to compare the cases positive for HPV 16/18 or non-16/18 as classified to the histologic diagnosis.

Results: Of the 130,648 Pap tests, 6,881 were classified as ASC-US and 142 as AGC, with 584 cases of ASC-US and 35 cases of AGC having Cobas HPV testing and histology follow-up including 3 cases with endometrial adenocarcinoma. The sensitivity and specificity of Cobas HPV test for detecting high-grade intraepithelial lesion (HSIL) or greater were 91% and 25% in ASC-US respectively, and 75% and 74% respectively in AGC. The Cobas HPV+ rates were significantly higher in women with CIN2+ and AIS in follow-up biopsy than those with benign or low grade lesions ($p<0.05$). Although HPV 16/18 and non-16/18 genotypes were associated with similar rates of dysplastic lesions (LSIL and above), HPV 16/18 infection was more significantly associated with high-grade lesions than non-16/18 ($p<0.05$).

Follow-Up Biopsy	Benign	LSIL	HSIL/AIS/Carcinoma	Total
ASC-US HPV+	149	238	64	451
ASC-US HPV-	85	42	6	133
AGC HPV+	5	1	8	14
AGC HPV-	12	5	4	21
Total	251	286	82	619

Conclusions: High-risk HPV genotypes, especially HPV 16/18 are strongly associated with HSIL and AIS on follow-up biopsies. Mixed infection with HPV 16/18 and non-16/18 genotypes has similar rate of high-grade lesions on follow-up as those infected by HPV 16/18 alone. Although the sensitivity of Cobas HPV test for HSIL is 91%, nearly 1 out of 10 tests are false negatives. This questions the effectiveness of using Cobas test for primary screening. Also, HPV testing should not be used alone in cases of AGC or for triage as high grade cervical lesions or endometrial malignancies will be missed. Co-testing with Pap and HPV is a prudent strategy till more data is available.

333 Utility of Peritoneal Washing Cytology in Staging and Prognosis of Ovarian and Fallopian Tube Neoplasms: A 10-Year Retrospective Analysis

Whitney Davidson, Rashna Madan, Maura O'Neil, Ossama Tawfik, Fang Fan. University of Kansas Medical Center, Kansas City, KS.

Background: The prognostic significance of peritoneal washing cytology in gynecologic malignancies is controversial. Some studies indicate that positive washings correlate with a worse prognosis while others suggest no change. The presence of malignant cells in peritoneal washings is currently part of the staging criteria of the AJCC TNM staging system in cases of ovarian and fallopian tube neoplasms without metastasis beyond the pelvis. Once the cancer has spread to local lymph nodes or beyond the pelvis, cytology has no impact on staging. In this study, we retrospectively reviewed all cases of ovarian and fallopian tube neoplasms in which cytological studies were performed. The utility of cytology in tumor staging and the relationship between cytology results and patient outcome are studied.

Design: All cases of ovarian and fallopian tube neoplasms in our institution between July 2002 and July 2012 were reviewed. Primary tumor characteristics including type, size, and pelvic extension were collected, categorized, and correlated with peritoneal washing cytology. Final tumor staging was reviewed and the impact of positive cytology was evaluated.

Results: 120 cases of ovarian and fallopian tube neoplasms without extra-pelvic metastasis were identified within the study period.

Tumor Type	Total (n)	Cytology Results		Upstaged by *P+	
		*P+	*P-	Yes	No
Mucinous Borderline Tumor	30	2	28	1	1
Serous Carcinoma	28	11	17	11	0
Serous Borderline Tumor	23	6	17	5	1
Endometrioid Carcinoma	10	3	7	1	2
Mucinous Carcinoma	9	2	7	1	1
Clear Cell Carcinoma	7	2	5	2	0
Mixed Epithelial Carcinoma	6	0	6	0	0
Seromucinous Borderline Tumor	3	1	2	1	0
Neuroendocrine Carcinoma	2	2	0	2	0
Granulosa Cell	1	0	1	0	0
MMMT	1	0	1	0	0

*P=Peritoneal Washing

Peritoneal washing cytology was positive in 24% (29/120) of neoplasms and upstaged the tumor 83% (24/29) of the time when positive. Overall, 20% (24/120) of the cases were upstaged based on positive cytology results.

Conclusions: Peritoneal washing cytology remains a useful staging tool for ovarian and fallopian tube neoplasms limited to the pelvic cavity. Positive cytology results in upstaging of all tumor types, but the frequency of positive washings varies depending on the type of tumor. Future studies will analyze follow-up data to determine if upstaging based on positive cytology adversely affects outcome.

334 β-Catenin Expression in Oropharyngeal Squamous Cell Carcinomas: Comparison and Correlation With p16 and HPV ISH

Drew Davis, Zachary Chinn, Cynthia Cohen, Momin Siddiqui. Emory University School of Medicine, Atlanta, GA.

Background: The importance of human papilloma virus (HPV) in the development of oropharyngeal squamous cell carcinoma (OSCC) has been increasingly recognized within the last few years. Numerous studies have demonstrated the improved prognostic significance of HPV positivity in OSCC. Recently the Wnt/β-catenin signaling pathway has been noted to be upregulated in head and neck cancers and several studies have identified mechanisms whereby HPV oncoproteins may induce nuclear β-catenin localization. The purpose of this study is to evaluate the expression of β-catenin in OSCC's as well as investigate its utility as an adjunct to p16 immunohistochemistry in the diagnosis of HPV.

Design: 68 cases of OSCC were identified and selected for this study. β-catenin (Santa Cruz Biotech, Santa Cruz, CA) IHC, p16 IHC, and HPV ISH were performed on 5 micron, formalin-fixed, paraffin-embedded sections, including fine needle aspiration cell blocks. Increased nuclear expression of β-catenin was considered positive. p16 exhibited nuclear and cytoplasmic staining and greater than 70% staining was considered positive, while HPV ISH staining was noted in nuclei in a dot-like pattern. HPV ISH was used as the gold standard.

Results:

Number of Cases	ß-catenin positive	p16 positive	HPV ISH positive
68	36/68 (52.9%)	42/68 (61.7%)	48/68 (70.6%)

Chi squared analyses demonstrated a strong correlation ($p<0.0005$) between p16 IHC and HPV ISH but no significant correlation between β-catenin and HPV ISH.

	HPV ISH Positive	HPV ISH Negative	
β-catenin Positive	26	10	PPV - 72.2%
β-catenin Negative	22	10	NPV - 31.2%
	Sensitivity - 54.2%	Specificity - 50.0%	

Conclusions: Increased nuclear β-catenin localization was observed in slightly over half of our cases. However, p16 yielded a higher specificity for diagnosing OSCC. Our data show that some OSCC's have abnormalities in the Wnt/β-catenin signaling pathway in conjunction with HPV positivity. While these abnormalities are not prevalent enough to warrant usage of β-catenin as an adjunct to p16 or HPV ISH in the diagnosis of HPV infection, they confirm the recent findings that HPV may induce β-catenin nuclear localization. Although of limited diagnostic utility, the presence of β-catenin overexpression may play a role in future directed therapies.

335 Triple Test for Patients With Thyroid Nodules By Cytopathologists

Luis De Las Casas, Stacy Molnar, Richard Cantley. The University of Toledo Medical Center, Toledo, OH.

Background: Ultrasound guided fine-needle aspiration (USFNA) is commonly performed for evaluation of patients with thyroid nodules. This procedure is performed by different physicians including endocrinologists, surgeons, radiologists and cytopathologists (CP). It is accepted that FNA accuracy is highest when the same physician examines the patient, performs the procedure, prepares the smears and does the microscopic evaluation. This study evaluates CP performed USFNA of thyroid nodules applying on site clinical, ultrasound and microscopic correlation (triple test).

Design: Thyroid USFNAs performed and interpreted by the same CP using on-site triple test between October 2010 and January 2014 were retrospectively reviewed. The aspirated material was obtained using a combination of Swedish and American methods with a 25 G x 1½ needle. Smears were stained for immediate evaluation with Diff-Quik. Additional smears were fixed in alcohol for Papanicolaou stain. Preliminary interpretations were incorporated in our clinical portal computer system within 2 hours of the procedure. The aspiration technique and number of passes were customized based on clinical and ultrasound features, and microscopic evaluation. Adequacy, complications, Bethesda diagnostic category and histologic correlation were analyzed.

Results: A total of 300 thyroid US-FNAs from 200 patients were evaluated. 299 samples were adequate for evaluation (99.7%). The minor complication of a small hematoma occurred in 2 cases (0.6%). Of the 300 cases, 251 (84%) were benign, 10 (3.3%) were malignant, 38 (12%) indeterminate [including 35 (11.6%) atypical/borderline, and 3 suspicious (1%)]. 17 of 251 (7%) cytologically benign cases had tissue correlation, all which were histologically confirmed as benign. Of the 35 atypical/borderline cases, 14 (40%) had tissue correlation, with 4 cases of nodular hyperplasia, 9 follicular adenomas, and 1 metastatic leiomyosarcoma. Among suspicious cases, 2 of 3 had histologic correlation (67%) and represented radiation changes from the same patient. Of the 10 malignant cases, 9 (90%) had tissue correlation. In all 9 cases the cytologic diagnosis was confirmed, including 1 case of metastatic leiomyosarcoma and 8 cases of papillary thyroid carcinoma. The negative predictive value for benign was 100%, and the positive predictive value for malignant was 100%. A final diagnosis was reported in 96% of the cases within 2 business days with most (75%) reported within 1 business day.

Conclusions: Our study shows that CP performed thyroid USFNA coupled with the onsite triple test is a safe, fast, and accurate diagnostic modality.

336 Methylation Markers of Pancreatic Carcinoma and Their Usefulness in Pancreatic FNA Cytology

Georgios Deftereos, Qinghua Feng, Melissa Upton, Joshua Stern, Stephen Hawes, Nancy Kiviat. University of Washington, Seattle, WA.

Background: Pancreatic cancer (PC) is the 4th leading cause of cancer-related death in the US. Modern imaging techniques have led to increasing numbers of pancreatic lesions detected; however, core biopsies are not routinely performed in this setting due to associated risks, including pancreatitis. Thus, endoscopic ultrasound-guided fine needle aspiration (EUS-FNA) is the preferred approach. Nonetheless, while cytology has a high positive predictive value for PC, its negative predictive value is low, due to sampling errors and inability to distinguish well-differentiated PCs from reactive changes. We hypothesize that the addition of methylation markers to cytology can help increase its sensitivity and specificity.

Design: We first used histologic material to determine which genes are frequently methylated in PC. We then examined whether methylation of these genes could be identified using FNA obtained material. 51 pancreatic resection specimens, including 16 pancreatic ductal adenocarcinomas (PDAC) and 35 benign (28 normal, 7 reactive) and 34 pancreatic FNA specimens, including 24 histologically confirmed as PDAC and 10 benign (by subsequent histology or negative follow-up) were examined by quantitative real-time PCR for promoter hypermethylation of APC, CCND2, CDH13, CDKN2A, IGSF4, RASSF1, SFN and UCHL1, genes previously reported to be methylated in PC.

Results: In resection specimens, UCHL1, CDH13 and CDKN2A were found methylated in PC (75%, 56% and 19% respectively) but never in benign (normal/reactive) specimens. 86% of PCs had one or more of these 3 genes methylated. CCND2 was found more often at high-level methylation (≥4% of gene copies) in PC, compared to benign specimens (56% vs 9%, p=0.0002), while high-level methylation of IGSF4 was found in 19% of PC, but not in benign specimens. In FNA material, methylation of CCND2 and UCHL1 (at any level) was found in 63% and 58% of PC cases respectively, but never in benign cases (p=0.007 and p=0.004 respectively).
Conclusions: 1. UCHL1, CDH13 and CDKN2A show promoter hypermethylation in PDAC but not in pancreatitis and normal pancreatic tissues.
2. Pancreatic FNA cytology specimens showed that methylation of UCHL1 and CCND2 were found in the majority of PDAC, but not in benign (negative/reactive) cases in this series.
3. Pancreatic FNA material can be used to detect methylation markers for PDAC, with results correlating to subsequent histology diagnosis.
4. These interesting results need to be studied in a larger series and to include other subtypes of PC.

337 Evaluation of Ki-67 in Pancreatic Neuroendocrine Tumors By Fine Needle Aspiration Biopsy With Automated Image Analysis

Dikson Dibe Gondim, Brian Collins, Laura Adhikari. Washington University School of Medicine, St. Louis, MO.

Background: Pancreatic neuroendocrine neoplasms are a rare group of tumors that represent only 1-2% of pancreatic tumors. Ki-67 immunohistochemistry (IHC) staining has been incorporated as a part of the WHO grading system for pancreatic resection specimens due to prognostic significance in patients with these lesions. The purpose of this study is to compare the performance of Ki-67 immunohistochemical positivity on fine needle aspiration (FNA) biopsy cell block specimens with follow-up surgical resection specimens using automated image analysis.
Design: A database search was performed using "neuroendocrine" or "endocrine" key words in the final diagnosis for pancreatic surgical and fine needle aspirates specimens. A total of 35 patients with a FNA cell block and follow-up surgical resection specimens were identified. Ki-67 IHC stains were then evaluated for both types of specimens using automated image analysis (Ventana software).
Results: Of the 35 correlation sets, fourteen cytology specimens differed in WHO grading from the surgical specimens. Of these, six specimens increased from WHO grade 1 on the cytology to WHO grade 2 on surgical resection. Eight specimens decreased in WHO grade with 3 cases having a WHO grade 2 on cytology, but WHO grade 1 on surgical resection and 5 cases with WHO grade 3 on cytology to WHO grade 2 on resection. Of the specimens in which the WHO grading matched , 11 specimens were a WHO grade 1 and 10 specimens were a WHO grade 2. Six out of thirty-five specimens had less than 1000 cells available for Ki-67 digital assessment on cell block, yet; only one of those specimens had a different WHO grade than from the surgical specimen.
Conclusions: Assessment of Ki-67 positivity by FNA biopsy may not be an accurate predictor of the final WHO grading on the surgical resection. 14 of 35 (40%) FNA biopsy cases had a different WHO grade than those from the surgical specimen. When Ki-67 positivity was incongruent, only a single WHO grade difference was noted.

338 Non 16/18 Human Papillomavirus Genotypes in Gynecologic Cytology Specimens

Jessica Dillon, Heather Steinmetz, Terri Wilson, Vijayalakshmi Padmanabhan, Laura Tafe. Dartmouth-Hitchcock Medical Center, Lebanon, NH.

Background: The current cytology guidelines recommend genotyping gynecologic cytology specimens for HPV genotypes 16 and 18, the two most commonly associated with cervical cancer. Available testing methodologies allow for genotyping of HPV 16 and 18 as well as a pool of "other" high risk genotypes (HR-HPV). The clinical significance and the occurrence rate of these "other" HR-HPV have not been well characterized. The aims of this study are to review our cytology genotyping experience and evaluate a set of "other" HR-HPV specimens to determine the distribution of "other" genotypes.
Design: HPV testing of ThinPrep® liquid based Pap test cervical specimens is performed on the Roche Cobas 4800 system either as a reflex test or concurrent with the Pap test. The Roche Cobas assay determines a negative or positive result and is able to report genotype results as types 16, 18 or "other" which is inclusive of a pool of 12 additional HR-HPV. Forty residual cytology specimens that typed as "other" were further genotyped using the Roche Linear Array HPV Genotyping test, a PCR-based line-blot assay. The test detects 37 high and low risk HPVs.
Results: Our laboratory began reporting cytology genotyping results from the Roche Cobas system in January 2013. Since that time we have tested 15,371 specimens with a 12.4% HR-HPV positivity rate. Cases reported as "other" HR-HPV comprised 73.6% of all positive results. In the HPV 16 or 18 positive cases, 29.8% harbored more than one HR-HPV. Of the 40 "other" HR-HPV samples that were further genotyped, the corresponding cytology diagnoses were: LSIL (7), ASC-US (15) and NILM (18). Thirty-six (90%) contained at least one HR-HPV (40% contained 2 or more), and in 20/40 (50%), a low-risk genotype was also detected. Four cases were negative for HR-HPV, possibly representing false positives on the Cobas assay. The most common "other" HR-HPV were 51, 52, 31, 59, 66, and 45 (all in 5 or more cases).
Conclusions: In our population, we found that 12.4% of tested cytology samples were positive for HR-HPV and the vast majority of these (73.6%) consisted of HR-HPV "other" than 16/18. The clinical significance of this finding is uncertain but important to further elucidate especially as primary HPV screening is adopted. Future studies will include correlation with HPV vaccination history, cytological diagnosis and follow-up colposcopy and biopsy results.

339 Tumor Heterogeneity Detected By EGFR Expression in Pancreatic Adenocarcinoma: Comparison of Endoscopic Ultrasound Guided Fine Needle Aspiration (EUS-FNA) Cell Blocks and Corresponding Resection Specimens

Rossitza Draganova-Tacheva, Phoebe Holmes, Marluce Bibbo, Ruth Birbe, James Casey, Raymond O'Neill, Stephen Peiper, Charalambos Solomides. Thomas Jefferson University Hospital, Philadelphia, PA.

Background: Expression of epidermal growth factor receptor (EGFR) in pancreatic adenocarcinoma may be associated with poor prognosis. Often, the initial diagnosis is made by EUS- FNA. In this study, we evaluate EGFR expression and mutational status by immunohistochemistry (IHC) and EGFR-gene amplification by Dual ISH in pancreatic FNA cell blocks and correlate the results with corresponding surgical resection specimens.
Design: Twenty-seven (27) EUS-FNA cell blocks of pancreatic adenocarcinoma and corresponding resection specimens were stained with: 1) anti-EGFR (5B7) rabbit monoclonal antibody against the intracellular domain, 2) anti-EGFR (3C6) mouse monoclonal antibody against the extracellular domain, 3) anti-EGFR L858R (SP125) rabbit monoclonal antibody, 4) anti-EGFR E746-A750 del(SP111) rabbit monoclonal antibody and 5) EGFR Dual ISH probe (Ventana Medical systems, Inc, Tucson AZ) using the Ventana BenchMark Ultra IHC platform. Moderate to strong membrane IHC staining in at least 10% of tumor cells was considered positive. For the Dual ISH probe, the ratio of EGFR to chromosome 7 and the average number of EGFR gene copies were recorded (20 nuclei). The mutation/deletion antibodies were read as positive or negative.
Results: EGFR expression with anti-EGFR (5B7) and anti-EGFR (3C6) antibodies showed significant correlation in the resection specimens. It was observed in 21 of 27 (77%) of the cases for both antibodies (Spearman Rank Correlation (ρ) =1). Heterogeneity of expression was present. The cell blocks did not show significant correlation (ρ=0.65). Expression was detected in 9 of 27 (33%) using anti-EGFR (5B7) and in 19 of 27 (70%) using anti-EGFR (3C6). Correlation between the cell blocks and resection specimens for both antibodies was not significant (ρ=0.63 and ρ=0.56). EGFR amplification was not observed in either the cell blocks or resection specimens. The EGFR gene copy: chromosome 7 ratio ranged from 1.0 to 2.1. The EGFR gene copy number ranged from 2.0 to 5.7 indicating polysomy 7. None of the cytology or resection blocks demonstrated EGFR L858R mutation or EGFR E746-A750 deletion.
Conclusions: EGFR protein expression is common in pancreatic adenocarcinoma. Both anti-EGFR (5B7) and anti-EGFR (3C6) are comparable in resection specimens. The discordance between cell blocks and corresponding tissue sections is likely due to tumor heterogeneity. EGFR L858R mutation, EGFR E746-A750 deletion and EGFR amplification are rare events in pancreatic adenocarcinoma.

340 Value of Ultrasound Guidance in Cytopathologist Performed Fine Needle Aspirations

Julie Dueber, Kurt Bernacki, Scott Kantola, Judy Pang, Madelyn Lew, Xin Jing, Amer Heider, Robertson Davenport, Michael Roh. University of Michigan, Ann Arbor, MI.

Background: Fine needle aspirations (FNAs) are routinely performed by cytopathologists (CPs) on palpable masses without ultrasound (US) guidance. Nonetheless, variations in the actual depth of palpable masses lead to occasional challenges. US guidance allows the CP to visualize the mass and guide needle placement during the FNA. This study retrospectively addressed the utility of US by comparing FNAs performed by CPs on palpable masses with and without US guidance.
Design: The pathology laboratory information system was searched to identify CP performed FNAs with and without ultrasound guidance from 3/2013 to 7/2014. The number of passes, location of the lesions, and diagnoses documented in the reports were recorded. The cytologic interpretations were categorized as either non-diagnostic (ND) or diagnostic (i.e., negative, atypical, suspicious, and positive for malignancy). Available slides were reviewed to determine the proportion of passes that contained diagnostic cellular material and cases in which diagnostic cellular material was present on the first needle pass.
Results: A total of 134 non-US-guided FNAs and 118 US-guided FNAs were analyzed. The number of ND cases was significantly higher for the non-US-guided FNAs [17/134; 12.7%] than for the US-guided FNAs [3/118; 2.5% (Fisher exact test, p = 0.004)]. The average number of needle passes per case was significantly greater for the non-US-guided FNAs (3.6) than the US-guided FNA cases (2.9; t-test, p = 0.0002). 22/118 (18.6%) of US-guided FNAs and 6/134 (4.5%) non-US-guided FNAs were completed after only a single pass (chi-square test, p = 0.0008). Excluding cases in which the slides were not available for review and excluding FNAs of simple cysts and seromas (2 US-guided, 1 non-US-guided) due to a lack of cellularity, all slides for 118 non-US-guided and 114 US-guided FNA cases were reviewed. Diagnostic material was present on the first pass in a significantly higher percentage of US-guided FNAs [81/114; 71%] than non-US-guided FNAs [68/118; 57.6% (chi-square test, p = 0.046)]. The percentage of passes with diagnostic material (# diagnostic passes/total # passes) was significantly higher for the US-guided FNAs [209/284; 73.6%] than for the non-US-guided FNAs [230/386; 60% (chi-square test, p = 0.0002)].
Conclusions: For palpable masses, US-guidance adds value to CPs in obtaining diagnostic cellular material more often on the first pass and with fewer passes overall than by palpation alone. This has a potentially beneficial impact on patient care owing to the increased precision and accuracy of needle guidance with ultrasonography.

341 Reliability of the Distinction Between Polyomavirus Infection (PyV) and High-Grade Urothelial Carcinoma (HGUC) in Urinary Tract Cytopathology (UTCy)

Reza Eshraghi, Stefan Pambuccian, Grazina Chatt, Zulfia McCroskey, Eva Wojcik, Guliz Barkan. Loyola University Medical Center, Maywood, IL.

Background: Cytologic characteristics of PyV in UTCy have been known since the late 1950's. "Decoy cells" have been described as a potential pitfall. The current efforts to standardize urinary tract cytology reporting ("The Paris System") have raised once again the question of how reliable the distinction between PyV and HGUC in UTCy is. The aim of this study was to determine how often an accurate distinction between PyV and HGUC can be made and the accuracy of UTCy PyV diagnosis.

Design: 2 cytotechnologists(CTs), 2 trainees(TRs), and 5 cytopathologists(CPs) reviewed 50 ThinPrep® UTCy cases previously diagnosed as HGUC(n=15), suspicious for HGUC(SHGUC, n=15), PyV cytopathic changes(n=20). All slides were re-stained with SV40 immunostain(CellMarque, clone MRQ-4). The interobserver concordance for PyV diagnoses and the accuracy rates for PyV compared to SV40 were calculated.

Results: Overall agreement, after combining SHGUC and HGUC categories was substantial (K=0.67) with interobserver kappa values of 0.42-0.88. Agreement between CT was highest (k=0.65-0.88).

Observers	1	2	3	4	5	6	7	8	9	10
1		0.72	0.83	0.651	0.589	0.754	0.722	0.584	0.793	0.584
2	0.72		0.88	0.839	0.642	0.722	0.84	0.485	0.683	0.643
3	0.83	0.88		0.724	0.676	0.757	0.801	0.592	0.797	0.674
4	0.651	0.839	0.724		0.574	0.651	0.758	0.427	0.615	0.504
5	0.589	0.642	0.676	0.574		0.672	0.642	0.75	0.711	0.667
6	0.754	0.722	0.757	0.651	0.672		0.801	0.584	0.793	0.75
7	0.722	0.84	0.801	0.758	0.642	0.801		0.564	0.683	0.643
8	0.584	0.485	0.592	0.427	0.75	0.584	0.564		0.706	0.491
9	0.793	0.683	0.797	0.615	0.711	0.793	0.683	0.706		0.538
10	0.584	0.643	0.674	0.504	0.667	0.75	0.643	0.491	0.538	

Of the 50 cases, SV40 stained 19/20 of the cases originally diagnosed as PyV; 3/15 cases originally diagnosed as SHGUC. Agreement between observers on cases originally diagnosed as PyV was 40-100% (mean 84.2%); 10-way agreement was achieved in 9/20 cases. Cases that stained for SV40 showed 20-100% agreement(mean 83.9%); cases originally diagnosed as SHUC had agreement for PyV diagnosis ranging from 70-100%. When compared to SV40 staining, the sensitivity was highest for CT (87.5-95.8%), the specificity highest for CP(80.8-92.3%).

	TR1	TR2	CT1	CT2	CT3	CP1	CP2	CP3	CP4	CP5
Sensitivity (%)	75.00	62.50	91.70	87.50	95.83	79.17	79.17	87.50	75.00	70.83
Specificity (%)	88.50	84.60	84.60	92.31	73.08	92.31	92.31	80.77	92.31	92.31
PPV (%)	42.00	38.00	52.00	91.30	76.67	90.48	90.48	80.77	90.00	89.47
NPV (%)	58.00	62.00	48.00	88.89	95.00	82.76	82.76	87.50	80.00	77.42

Conclusions: Our study found that agreement for the diagnosis of PyV in UTCy is substantial to almost perfect. However, occasional cases of PyV were misclassified as SHGUC by some observers, suggesting the use of intradepartmental consultation or consensus review of SHGUS cases.

342 Fine-Needle Aspiration Biopsies of Thyroid Nodules Classified With the New Italian System in Comparison With the Bethesda Reporting System: Preliminary Results of an Institutional Series

Guido Fadda, Esther D Rossi, Patrizia Straccia, Tommaso Bizzarro, Domenico Damiani, Chiara Brunelli, Celestino Pio Lombardi, Alfredo Pontecorvi. Catholic University, "Agostino Gemelli" School of Medicine, Rome, Italy.

Background: Up to 5% of the general population has a palpable thyroid nodule, though only 5% of them actually harbour malignancy. FNAC (fine-needle aspiration cytology) is the only diagnostic test that can provide a definitive preoperative diagnosis of malignancy. The aim of this study is to compare an institutional series classified according to the new Italian reporting system with the Bethesda System for Reporting Thyroid Cytopathology (TBSRTC). The latest 6-tiered revision of the Italian system, which is similar to TBSRTC, includes the following categories: TIR1- non diagnostic; TIR1C- cystic; TIR2- negative for neoplasia; TIR3A– low-risk indeterminate lesion; TIR3B– high-risk indeterminate lesion; TIR4- suspicious for malignant neoplasm; TIR5- positive for malignancy.

Design: From April until September 2014, 450 patients underwent an US-guided FNAC. The specimens were processed by liquid-based cytology (LBC- Hologic Co. Marlborough, MA) and diagnosed according to the Italian reporting system. The resulting frequency of each category was compared with the TBSRTC suggested rates.

Results: The 450 samples were classified as follows: TIR1=18 (4%); TIR1C=12 (2.6%); TIR2=334 (74.2%); TIR3A=21 (4.6%); TIR3B=22 (4.8%); TIR4=13 (2.8%); TIR5=30 (6.6%). Thirty-four patients underwent surgery with 12 (35,3%) malignant neoplasms at histology. These frequency rates are in agreement with the suggested ranges of the Bethesda system. Though the number of histological controls is limited, the TIR3B category was virtually represented by oxyphilic neoplasms (HCN, 17 cases: 77%) 2 out of them operated with a diagnosis of adenoma.

Conclusions: This preliminary study emphasizes the substantial reproducibility of the two reporting systems. Although the histological follow-up is still limited, the low rate of malignancy for the category of TIR3B in the surgical series is probably due to both the difficulty in the application of the diagnostic criteria for this category and to the high rate of HCN, which generally result as benign at histology. In conclusion the recently updated Italian reporting system can be considered comparable to TBSRTC and may represent the first step towards a globally shared reporting system for thyroid cytopathology.

343 Cytologic, Histologic and Molecular Features of Primary Thyroid Neoplasms With Signet-Ring Cells

Nada Farhat, Zhengtong Pei, Jeffrey Krane, Peter Sadow, William Faquin. Massachusetts General Hospital, Boston, MA; Barnabas Health, Livingston, NJ; Brigham & Women's Hospital, Boston, MA; Harvard Medical School, Boston, MA.

Background: We have investigated a group of primary thyroid neoplasms containing cells with prominent intracytoplasmic vacuoles producing a signet-ring configuration, a finding that may be a diagnostic pitfall by FNA biopsy. We describe a series of twelve cases of primary thyroid neoplasms with signet ring cells (SRC), including both the original cytology and subsequent excision, the largest series to date.

Design: A total of 12 thyroid neoplasms with SRC were identified from the archives of the MGH and BWH from 2000-2014. Seven cases of follicular adenoma and five cases of papillary thyroid carcinoma were studied. Correlation of FNA with thyroid surgical resection specimens was performed. SRC were evaluated with mucicarmine, Periodic Acid-Schiff stain with or without diastase digestion and Alcian blue and immunohistochemically for thyroglobulin and BRAFVE. Major thyroid molecular alternations, including BRAF, RAS, RET/PTC, and PAX8/PPARγ, are also studied.

Results: The 12 cases were from 2 men and 10 women with a mean age of 53 (age range: 17-81). The average size of the neoplasms was 2.4 cm (range: 0.2-4.5 cm). The tumors with SRC included 7 follicular adenomas and 5 papillary thyroid carcinomas. 4 cases led to diagnostic difficulties in FNA samples where up to 50% of the tumors were comprised of SRC. Histochemical evaluation showed that in 10 cases the cytoplasmic vacuolization was due to accumulated thyroglobulin. Molecular analysis showed that all 4 PTC were negative for mutations in BRAF, and translocations were absent in one PTC case with the others currently being evaluated.

Conclusions: Primary thyroid neoplasms that contain SRC may be seen in both benign and malignant tumors, confounding FNA biopsy interpretation. Our analysis of these tumors, including the cytologic, histologic, histochemical, and molecular features of this under-recognized group of thyroid neoplasms, may allow for improved cytological risk stratification in this diverse group of entities.

344 Association of Morphology and EGFR Mutation in Lung Adenocarcinoma: Appraisal of 40 Cytology and Histopathology Cases

Jingyang Feng, Sharon Zhang, Ward Reeves, Richard DeMay. University of Chicago, Chicago, IL.

Background: Lung cancer is a leading cause of mortality, and patients often present at a late stage. Recently, identification of lung adenocarcinoma driver mutation, epidermal growth factor receptor (EGFR) has led to the implementation of targeted therapies. Testing for mutations in EGFR is therefore an important step in the treatment-decision pathway. This study aimed to examine morphological associations of EGFR mutation in cytology and histopathology specimens.

Design: Forty consecutive primary lung adenocarcinoma cases with available EGFR mutation testing in cytology, small biopsy and surgical resection specimens were selected from 2011 to 2014. EGFR mutation was detected by next generation sequencing technology. All the cases were classified by EGFR mutation results and separated into EGFR mutation positive and negative groups. X^2 test was used to evaluate possible statistical interrelationships between EGFR mutation and histological tumor grade. Furthermore, cytologic features like anisonucleosis, markedly increased N/C ratio, and presence of macronucleoli were observed in cytology specimens to investigate potential cytologic features associated with EGFR mutation.

Results: Among 40 lung adenocarcinomas with EGFR testing, EGFR mutation was identified in 9 cases (22.5%), of which 8 (88.8%) were diagnosed as well or moderately differentiated adenocarcinoma and 1 (11.2%) was poorly differentiated. While 20 out of 31 cases (64.5%) without EGFR mutation were poorly differentiated tumors. The percentage of high grade tumor in EGFR mutation group is significantly lower than that in the EGFR mutation negative group (p=0.007). Among the observed cytologic features, anisonucleosis was strongly associated with EGFR mutation negative cases

(p= 0.022), while there was no statistically significant difference in increased N/C ratio (p= 1.0) and presence of macronucleoli (p=1.0) between different EGFR status groups.
Conclusions: This study indicated that there is a strong inverse association between EGFR mutation and histopathologic grade, which may explain the favorable clinical outcome in patients with EGFR mutation. Anisonucleosis is the only observed cytologic feature which shows inverse association with EGFR mutation. Markedly increased N/C ratios and presence of macronucleoli were not significantly different between the two EGFR status groups.

345 CD56 Is a Sensitive Marker for Neuroendocrine Tumors in Endobronchial Ultrasound-Guided Fine Needle Aspiration (EBUS-FNA) Specimens

Yumei Fu, Chen Yang, Jeff Wang. Washington University School of Medicine, St. Louis, MO.

Background: Neuroendocrine tumors (NETs) include a spectrum of epithelial neoplasms with predominant neuroendocrine differentiation. In endobronchial ultrasound-guided fine needle aspiration (EBUS-FNA) specimens, the prognostic spectrum consists predominantly of pulmonary typical carcinoid, atypical carcinoid, small cell carcinoma, and large cell carcinoma. Immunohistochemistry markers have been proposed to aid the diagnosis of NETs, including CD56, synaptophysin, and chromogranin. Anti-CD56 recognizes two proteins of the neural cell adhesion molecule, expressed on most neuroectodermal derived cell lines and NETs. CD56 is a commonly used marker for NETs, however, studies of its utility in cytology specimens are not found in literatures. Thus we sought to perform a retrospective study to compare the three markers in EBUS-FNA specimens.
Design: We performed an institutional CoPath database search for neuroendocrine tumors from EBUS-FNA cytology materials during the 5-year period (2009-2014). We studied 69 NETs cases including low to intermediate and high grade according to the nomenclature and grading system of 2010 WHO and the North American Neuroendocrine Tumor Society (NANETS) guideline. These cases include 21 lung primary NETs, and 48 metastatic NETs from pulmonary regional lymph nodes. All the cases had the three markers performed. Focal or weak positivity were accounted as negative. The percentage of positivity of each marker was summarized in table 1.

	CD56	Synaptophysin	Chromogranin
Positive	63/69 (91.3%)	53/69 (76.8%)	35/69 (50.7%)
Negative	6/69 (8.7%)	16/69 (23.2%)	34/69 (49.3%)

Results: All three immunohistochemistry stains were useful in supporting the diagnosis of NETs, but the markers had distinctively variable positive staining patterns (table 1). CD56, synaptophysin, and chromogranin were positive in 63/69 (91.3%), 53/69 (76.8%), and 35/69 (50.7%), respectively. In all NETs in EBUS-FNA, CD56 showed statistically higher sensitivity than synaptophysin ($p = 0.0099$) and chromogranin ($p < 0.0001$). More interestingly, CD56 and synaptophysin positivity covered the whole spectrum of NETs in EBUS-FNA, no single case was positive only for chromogranin.
Conclusions: We recommend the use of CD56 and synaptophysin to aid the diagnosis of NETs in EBUS-FNA specimens.

346 Utility of Synaptophysin, Chromogranin, and CD56 Immunostains in the Diagnosis of Neuroendocrine Tumors in Cytology Specimens

Yumei Fu, Chen Yang, Jeff Wang. Washington University, St. Louis, MO.

Background: Neuroendocrine tumors (NETs) include a spectrum of epithelial neoplasms with predominant neuroendocrine differentiation, seen in a variety of organs. A number of immunohistochemistry markers have been proposed to aid the classification of NETs, including synaptophysin, chromogranin, and CD56 as the most common ones. However, the immunoprofiles of the three markers in cytology specimens have been lacking. Therefore, the aim of the retrospective study was to establish and compare the immunohistochemical features of the three markers in NETs from different anatomical sites for best utilization on cytologic materials.
Design: We performed an institutional database search for all neuroendocrine tumors from fine needle aspiration materials during 2009 to 2014. The cases included 123 NETs with low to intermediate (Well Differentiated) and high grade (Poorly Differentiated). There were 38 cases from gastroenteropancreatic NETs (GEP-NETs) (15 surgically confirmed including WD=11, PD=4), 69 lung and regional lymph nodes and 16 metastatic NETs from other sites. All the cases had the three mentioned markers performed. Focal or weak positivity were accounted as negative.
Results: Synaptophysin, chromogranin and CD56 were positive in 38/38 (100%), 37/38(97.37%) and 28/38 (65.79%) in all GEP-NETs. Synaptophysin and chromogranin showed similar detection rates, higher than CD56 in GEP-NETs, irrespective of grading. In lung and regional lymph nodes, the three markers detected immunoactivities in 53/69 (76.81%), 35/69 (50.72%) and 63/69 (91.3%) of cases, respectively. CD56 was the most sensitive in all three markers in those cases, among which 55 were small cell carcinoma. Synaptophysin and CD56 were equally positive in 11/14 (78.57%) of metastaic NETs at other sites, among which 10 were small cell carcinoma.
Conclusions: A preference of the markers can be given if the tumor volume is low on cell block. For lung and regional nodes, majority of cases are primary or metastatic small cell carcinoma of lung , CD56 is the most sensitive marker. Single use of CD56 or the combination of synaptophysin and CD56 is recommended. Synaptophysin, chromogranin or combination of the two is equally effective in pancreatic NETs, which are predominately low to intermediate grade. For metastatic NETs at other anatomical sites, synaptophysin, CD56 or combination of these two are equally effective.

347 Minimally Invasive Mediastinal Staging of Lung Cancer: How Accurate Is Endobronchial Ultrasonography-Guided Fine-Needle Aspiration Cytology?

Chen Gao, Saraswati Pokharel. Roswell Park Cancer Institute, Buffalo, NY.

Background: Mediastinal staging in patients with lung malignancy, particularly non–small cell lung carcinoma (NSCLC) is crucial in dictating surgical *vs.* nonsurgical treatment. Cervical mediastinoscopy-guided biopsy is the "gold standard" in mediastinal staging but is invasive and limited in assessing lower mediastinal and hilar lymph nodes. Less invasive approaches such as endobronchial ultrasonography-guided fine-needle aspiration (EBUS-FNA) have become more widely utilized for staging and documentation of recurrence. We investigated the accuracy of EBUS-FNA in mediastinal and hilar lymph node (LN) staging in our cancer center.
Design: We reviewed pathology reports of EBUS-FNA from October 2011 to September 2014. Corresponding results from the biopsy or resection of the same LN that had been sampled in EBUS-FNA within 6 months of the EBUS-FNA procedure were compared. The histology results from the biopsy or resection specimens were used as the gold standard for comparative evaluation of the cytology report.
Results: We identified a total of 298 patients who had undergone EBUS-FNA during the three-year period. Forty-eight LN from 39 patients had matching cytology and histology data. Of the 29 patients who had diagnoses of malignant disease in the LN by EBUS-FNA, most were metastatic lung carcinomas, including 22 NSCLC (75.9%), 3 small cell carcinomas (10.3%), two metastatic malignancy other than the lungs (6.9%), and two lymphomas (6.9%). The FNA samples from the LN of the remaining 10 patients were negative for malignancy. The staging of the mediastinal and hilar LN with EBUS-guided FNA has sensitivity of 89.6%, specificity of 94.7%, positive predictive value of 96.3%, negative predictive value (NPV) of 85.7% (Table 1).
Conclusions: EBUS-FNA is an effective and relatively accurate method in assessment of the status of mediastinal lymph nodes, and is both sensitive and specific in diagnosing metastatic disease involving mediastinal and hilar lymph nodes.
Table 1. Diagnostic accuracy of EBUS-FNA of mediastinal and hilar LN compared to the biopsy/resection.

	EUS-FNA
True Positive	26
True Negative	18
False Positive	1
False Negative	3
Sensitivity	26/29=89.6%
Specificity	18/19=94.7%
PPV	26/27=96.3%
NPV	18/21=85.7%

348 Cytospin of Formalin Fixative Enhances Giardia Morphology in Duodenal Biopsy Specimens

Nariman Gobara, Rana Hoda, Michael Chaump, Debra Beneck, Nicole Panarelli, Lihui Qin, Jose Jessurun, Rhonda Yantiss. Weill Cornell Medical College, New York, NY.

Background: Giardiasis is the most common parasitic infection in the United States. Symptoms are variable and infection produces minimal, if any, endoscopic or histologic changes, and thus, organisms can be easily overlooked in some cases. The purposes of this study were to determine whether Giardia could be isolated from the formalin fixative of biopsy samples and to evaluate the value of fluid analysis compared to histologic assessment of biopsy samples.
Design: We prospectively evaluated duodenal biopsy samples from patients with either a clinical suspicion of giardiasis or symptoms compatible with that diagnosis (e.g. diarrhea, bloating, abdominal pain). Biopsy samples were routinely processed and cytology slides were prepared from the formalin remaining in the container using standard Cytospin protocols. All the cytology slides were reviewed by a cytopathologist blinded to the biopsy findings.
Results: 59 duodenal biopsies and paired Cytospin slides were included in the study. Histologic diagnoses included duodenal giardiasis (2 cases, 3%), normal findings (38 cases, 64%), peptic injury or active duodenitis (13 cases, 22%) and intraepithelial lymphocytosis or celiac disease (6 cases, 9%). Ten cases showed detached degenerated epithelial cells or mucus droplets in the intervillous space that resembled Giardia. Cytologic evaluation of Cytospins revealed organisms in the same two cases that were positive by biopsy analysis, although Giardia was more numerous in the cytology preparations (500 and 170 trophozoites) compared with tissue samples (100 and 50 trophozoites) with better preserved morphological features (figure:1). All of the remaining Cytospin slides were negative for Giardia.

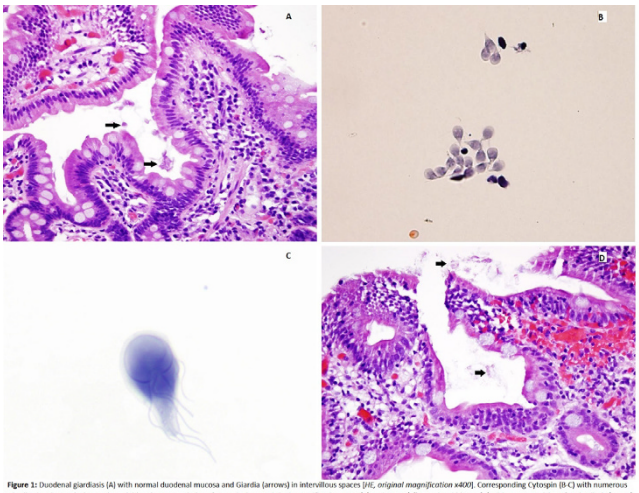


Figure 1: Duodenal giardiasis (A) with normal duodenal mucosa and Giardia (arrows) in intervillous spaces (HE, original magnification x400). Corresponding Cytospin (B-C) with numerous Giardia showing typical pear shaped, binucleate morphology (Papanicolaou, original magnification x400 (B) and x1000(C)). Duodenal biopsy (D) with areas suspicious for giardiasis (arrow) (HE, original magnification x400).

Conclusions: The Cytospin protocol can be used to enhance detection of organism in the left-over formalin from biopsy samples. This technique yields far more organisms (three-fold increase) than are evident in tissue biopsy samples with superior organism morphology. It may also resolve diagnostic issues when tissue samples are considered "suspicious" for organisms. Future studies may demonstrate a broader role for cytological assessment using Cytospin technique in the evaluation of gastrointestinal diseases.

349 The Paris System for Urinary Cytology: Will It Be Useful for Patient Management?

Elvia Goez Gutierrez, Roberto Ruiz Cordero, Alia Gupta, Carmen Gomez-Fernandez, Monica Garcia-Buitrago, Merce Jorda. University of Miami, Jackson Memorial Hospital, Miami, Fl.

Background: The American urological association recommends urinary cytology for screening and evaluation of patients with urothelial carcinoma(UC). Different reporting systems have been used with poor standardization. The international academy of cytology and The American society of cytopathology at the international cytology congress in Paris 2013, presented the Paris system for reporting urinary cytology. The system is not well defined yet and includes the following proposed categories(PC): 1.Non diagnostic or unsatisfactory, 2.Negative for malignancy, 3.Atypical urothelial cells of uncertain significance (AUC-US), 4.Atypical urothelial cells suspicious for high grade UC (AUC-H), 5.Low grade UC, 6.High grade UC, 7.Other malignancies, primary vs. metastatic and 8.Ancillary studies.

Design: A retrospective review of urinary cytology reports with concomitant biopsy, TURB or resection pathology reports was undertaken. We reclassified the cytopathology reports based on our interpretation of the Paris System Categories. Cases interpreted as non-carcinoma were included to evaluate the sensitivity and specificity of PC 3 and 4, comparing with the concurrent histology.

Results: A total of 123 reports from 89 patients were reviewed. 81 reports (66%) were categorized as non-carcinoma (PC 1,2,3,4). The cases previously diagnosed as reactive/atypia were reclassified in as PC 2 and as PC 3 for comparison. When classified as PC 2, 7 cases were AUC-US, which showed UC on the concurrent biopsy, and 54 cases were negative, 30 of them showed UC on biopsy. When classified as PC3, 18 cases were classified as AUC-US, 10 of them showed UC and 8 were negative in the concurrent biopsy. 44 cases were classified as negative and 16 (36%) showed UC on histology. 18 cases were classified as AUC-H and all of them showed UC on histology.

	Reactive/Atypia as PC2	Reactive/Atypia as PC3
AUC-US sensitivity	12	17
AUC-US specificity	100	68
AUC-H sensitivity	32	32
AUC-H specificity	100	100

Statistica analysis.

Conclusions: The Paris category of AUC-US and AUC-H will improve the standardization of the urinary cytology reporting with practical information for the urologists. The category AUC-US could recommend closest follow up for cytologies previously diagnosed that have been diagnosed as reactive/atypical, interpreted by the urologist as negative. The category of AUC- H could suggest follow up with cystoscopy. Although the Paris System does not have established criteria for each category, if implemented, can yield a closer follow up for patients with UC. The system will facilitate the cytopathologist interpretation.

350 Multimodal Sampling of Pulmonary Lesions Yields Minimal Improvement in Sensitivity Compared To Transbronchial Needle Aspiration Alone: A Single Institution Review of 248 Cases

Lindsay Goicochea, Paul Staats. University of Maryland Medical Center, Baltimore, MD.

Background: Transbronchial needle aspiration (TBNA) is an integral tool for the diagnosis of pulmonary lesions and the staging of non-small cell lung cancers. Frequently, other sampling modalities (bronchial brushing, bronchial washing, tissue biopsy, and/or bronchoalveolar lavage (BAL)) are employed in addition to TBNA. There is little evidence on the added value of these other modalities in patients undergoing TBNAs, particularly those that include endobronchial ultrasound guidance (EBUS). Our study compares the efficacy of TBNA sampling of peribronchial lesions to that of additional modalities performed at the time of TBNA.

Design: Cases of malignant peribronchial lesion(s) with TBNA sampling, collected between January 2011 and June 2013 at our institution, were identified and the pathology reports were reviewed retrospectively. Data collected included the location of the lesion, the TBNA cytologic diagnosis, use of EBUS guidance or not, and the results of additional sampling modalities performed at the time of TBNA.

Results: 248 patients had TBNA sampling of 303 peribronchial lesions: 138 lymph nodes (46%), 8 endobronchial masses (3%), 156 lung masses (51%), and 1 site unspecified. EBUS guidance was used in 185 (61%). TBNA was positive for malignancy in 293 cases (96.6%; 97.3% of EBUS and 95.8% of non-EBUS). 183 lesions (60%) were sampled by other modalities: 119 (39.3%) had washings, 63 (20.8%) BAL, 63 (20.8%) brushings, and 43 (14.2%) tissue biopsy; 75 (41%) employed more than 1 additional modality. A positive diagnosis was made in 33 (76.7%) biopsies, 47 (74.6%) brushings, 26 (21.8%) washings, and 9 (14.3%) BALs. In 41 (22.4%) cases with multimodality sampling, TBNA provided the only malignant diagnosis. Of the 10 negative TBNAs, a definitive diagnosis of malignancy was attained by bronchial brushing in 5 cases (50%), by tissue biopsy in 1 case (10%), and by both brushing and tissue biopsy in 4 cases (40%).

Conclusions: In our institutional experience, TBNA is the best single modality for cytologic diagnosis of peribronchial malignancy. All other modalities displayed a substantially inferior yield to that of TBNA; bronchial brushings and tissue biopsies added very few additional definitive diagnoses, and bronchial washings and BALs added no diagnostic value in this study. Whether these modalities contributed to tumor typing or molecular testing was beyond the scope of this study. Clinicians should consider whether these additional modalities are necessary in patients undergoing TBNA.

351 Utility of Afirma Testing on Thyroid Fine Needle Aspiration (FNA) Specimens: "Suspicious" Results With Clinicopathologic Follow-Up

Adam Gomez, Christina Kong. Stanford Hospital and Clinics, Stanford, CA.

Background: Molecular testing for common genetic abnormalities is increasingly utilized for cases with indeterminate cytology on fine needle aspiration (FNA). Afirma is a Gene Expression Classifier (GEC) test that measures expression of 142 genes and interprets the findings as "benign" or "suspicious." The reported high negative predictive value of this GEC promotes follow-up instead of surgical intervention for patients with indeterminate FNA results and Afirma "benign." However, Afirma "suspicious" has poor positive predictive value. In this study, we reviewed our experience with Afirma, including clinical and pathologic follow-up.

Design: A pathology database search from 2/2013 to 8/2014 identified 73/1193 (6%) thyroid FNA specimens classified as indeterminate: atypia of undetermined significance (AUS), follicular lesion of undetermined significance (FLUS), suspicious for follicular neoplasm (SFN), suspicious for Hurthle cell neoplasm (SHN). Cytologic interpretation was performed at our institution and material was sent for Afirma testing based on our cytologic interpretation. 15/73 had material sent for Afirma testing. Clinicopathologic follow-up was obtained.

Results: Afirma results were available for 12/15 cases; 2 had inadequate RNA and 1 was damaged in transit.

FNA Diagnosis	Size (cm)	Afirma Result	Follow-up
AUS	1.0	Suspicious	Medullary thyroid carcinoma
AUS	5.9	Suspicious	Surgical referral
FLUS; SHN	2.3	Suspicious	Surgical referral
FLUS x2	5.9	Suspicious	Follicularcarcinoma, Hurthle cell variant
SFN	1.2	Suspicious	Follicular adenoma
SFN	4.6	Suspicious	Surgical referral
SFN	1.8	Suspicious	Surgical referral
SFN	2.4	Suspicious	Surgical referral
SFN	3.1	Suspicious	Follicular adenoma
SHN	4.1	Suspicious	Follicular adenoma with Hurthle cell change
Favor BTN (prior FLUS)	2.1	Suspicious	Surgical referral
Squamous cyst	3.9	Suspicious	Follow-up ultrasound

Conclusions: 12/12 cases were interpreted as "suspicious" by Afirma and 11/12 were referred for surgery. While GEC tests may provide a management benefit in practices without fellowship-trained cytopathologists, the role of such tests in specialized practices

may be limited given that Afirma results did not change management in our set of cases. None of the cases received a "benign" Afirma result which would allow for clinical follow-up and avoidance of surgery.

352 Focal Squamous Atypia and Unsatisfactory Specimens Are Responsible for Significant Proportion of False Negatives in Anal Cytology

Abha Goyal, Tarik Elsheikh. Cleveland Clinic, Cleveland, OH.

Background: Similar to cervical cytology (CC), anal cytology has been proposed as a screening test for the early detection of high-grade anal intraepithelial neoplasia (HGAIN), especially in high-risk populations. Whereas cytologic and adequacy criteria are well described in CC, they are not well established for AC. We recently reported that 33% of NILM diagnosed by AC were found to have HGAIN on follow-up biopsies (FUB). Whereas epithelial abnormalities in AC are usually aggressively pursued, NILM patients maybe lost to follow-up or may undergo repeat cytology. We conducted this study to identify cytologic or adequacy features that may contribute to reducing the false negative (FN) rate associated with NILM in anal cytology.

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Design: A retrospective database search was conducted over a 5.5 year period for all NILM cases with FUB of at least HGAIN (group A). All NILM cases with negative FUB were also retrieved as control slides (group B). Blinded to FUB, slides from both groups were randomly mixed, and re-evaluated. Several cytologic features were examined, including glandular/metaplastic cells, keratosis, squamous adequacy, and atypia.

Results: 8/20 patients in Group A (40%) had revised second review diagnoses, including 4 ASCUS and 4 unsatisfactory. Glandular cells were rare to absent in 13/20 cases (65%), and >20 clusters in 5 patients (25%). In group B, there was only 1/13 revised diagnosis of unsatisfactory (7.6%). Glandular cells were rare to absent in 7 cases (54%), and >20 clusters found in 6 cases(46%). Keratosis and reactive atypia were present in both groups with similar frequencies.

Group	Original Cytology	Revised Cytology		
		NILM	ASC-US	Unsatisfactory
Group A (High-grade dysplasia or worse on follow-up)	NILM (n=20)	12	4	4
Group B (Negative follow-up)	NILM (n=13)	12	0	1

Conclusions: 40% of FN diagnoses were revised to ASCUS or unsatisfactory. Glandular cells were more abundant in TN cases compared to FN, hinting that quantity may correlate with adequate sampling. This study suggests that vigilant search for focal atypia and strict application of adequacy criteria can significantly reduce FN rate in anal cytology.

353 Inter-Observer Agreement in Cytologic Grading of Atypia in Neoplastic Pancreatic Mucinous Cysts Using the Two-Tiered Approach

Abha Goyal, Fadi Abdul-Karim, Bin Yang, Jinesh Patel, Jennifer Brainard. Cleveland Clinic, Cleveland, OH.

Background: The accurate cytologic grading of epithelial atypia in fine needle aspirates (FNAs) of pancreatic neoplastic mucinous cysts has important implications for clinical management. The Papanicolaou Society of Cytopathology has recommended a two-tiered system of low-grade (LG) and high-grade (HG) for grading this atypia. To examine the inter-observer agreement using this approach, we asked a group of cytopathologists at our institution to qualify atypia in a selected group of pancreatic cysts.

Design: Twenty cases of pancreatic neoplastic mucinous cyst FNAs with documented histologic follow-up and representative lesional cells were randomly selected. Blinded to the histologic outcome, 4 cytopathologists were independently asked to assign the highest grade of atypia using the 2-tiered system of LG and HG atypia on these cases. 1-2 slides (Papanicolaou stained smears and/or ThinPrep slides) with 3-5 marked cell groupings were reviewed per case. The interobserver agreement was calculated using the kappa statistic.

Results: The overall interobserver agreement in grading of atypia was fair (k=0.25) with complete agreement in 12 cases (60%) and agreement between 3 participants in 6 cases (30%). Based on the histologic outcomes, cases were stratified into 2 groups - Group A (HG dysplasia or worse on follow-up) and Group B (LG or intermediate grade dysplasia on follow-up). Group A (n=12) showed moderate agreement (k=0.49) with a majority diagnosis (≥ 3 participants) of HG atypia in 11 cases. Group B (n=8) showed no agreement (k<0) with the majority diagnosis of HG atypia in 6 cases.

Histologic Diagnosis	Majority Diagnosis of High-grade Atypia (≥3 participants)	Majority Diagnosis of Low-Grade Atypia (≥3 participants)
High-grade Dysplasia or worse (n=12)	11	1
Intermediate-grade Dysplasia (n=2)	2	0
Low-grade Dysplasia (n=6)	4	0

Based on the cytologic grade rendered by the most experienced observer, the sensitivity and specificity of cytology in diagnosing high-grade dysplasia or worse was 91.7% and 62.5% respectively.

Conclusions: Our study shows that the cytologic recognition of HG dysplasia or worse as HG atypia in pancreatic mucinous cysts has a good degree of inter-observer reproducibility amongst cytopathologists. In contrast, the problematic area with lack of agreement appears to be the cytologic recognition of LG and intermediate grade dysplasia as LG atypia. There is a need for additional studies with development of reproducible criteria and educational tools to help in this challenging distinction.

354 Reproducibility of the Papanicolaou Society of Cytopathology Guidelines for Pancreatobiliary Cytology – Intraobserver and Interobserver Variability in 156 Samples

Rosario Granados, Carlos Plaza, Eva Tejerina, Susana Prados, Vicente Emilio, Alejandra Caminoa. Hospital Universitario Sanchinarro, Madrid, Spain.

Background: There is a changing scenario in the clinical management of patients with pancreatobiliary tumors, with some entities requiring immediate surgery and premalignant and cystic lesions benefiting from a conservative approach. The role of the cytopathologist in these lesions is essential. Recently, a six-tiered standardized terminology has been proposed. In order to implement these guidelines, intra and interobserver variability studies are needed.

Design: Clinical, radiological, biochemical information and all cytology smears from a total of 156 samples, including 18 biliary tree brushigs and 138 endoscopic ultrasound (EUS)-guided pancreatic FNAs, were reviewed by two expert cytopathologists in a blinded study. Intraobserver and interobserver variability for cytological diagnosis was analyzed using kappa statistics for the unmodified six-tiered approach. A panel of 23 cytological diagnostic criteria was applied for each case. Clinico-pathological correlation was performed.

Results: There were 77 patients with histological correlation and 70 additional cases with a reliable clinical diagnosis. Four suspicious or malignant diagnoses and one case diagnosed as pancreatic neuroendocrine tumor (PanNET) had negative histology (false positives). There were 2 false negative diagnoses in carcinomas (0,25%). From 8 serous cytadenomas, 4 (50%) were reclassified from non-diagnostic to benign neoplastic lesions. Seven of 20 mucinous tumors (35%) had their diagnoses changed following the proposed nomenclature. Apart from high grade tumors, PanNET was the most feasible neoplasm to diagnose applying cytological criteria alone. Intraobserver agreement before and after using the proposed nomenclature had a kappa value of 0,75. The value for interobserver agreement for the 6-tiered classification was 0,63.

Conclusions: The standardized terminology for pancreatobiliary cytology recently proposed by the Papanicolaou Society, shows an acceptable intra and interobserver variability using a very stringent approach. The use of this nomenclature reduces the non-diagnostic diagnoses in cystic lesions. A joint multidisciplinary effort is needed to achieve the adequate patient management in these lesions.

355 Follicular Neoplasm, Hürthle (Oncocytic) Cell Type Versus Typical Follicular Neoplasm on Fine Needle Aspiration: Differences in Risk of Malignancy and Mutational Profiles

Christopher Griffith, Jenna Wolfe, Linwah Yip, Shane LeBeau, Sally Carty, Marina Nikiforova, Yuri Nikiforov, N Ohori. University of Pittsburgh Medical Center, Pittsburgh, PA.

Background: The Bethesda System for Reporting Thyroid Cytopathology estimates malignancy risk for the follicular neoplasm (FN) category of 15-30%. FNs comprised of predominantly Hürthle cells are reported as Follicular Neoplasm, Hürthle (Oncocytic) Cell Type (FNHCT) but malignancy risk for this category is unclear. Here we compare the risk of malignancy and mutational profiles of FNHCT and FN.

Design: Search of our laboratory information system between 9/2008 and 12/2013 returned 212 thyroid fine needle aspiration cases from 201 patients with the cytologic diagnosis of FNHCT. Of these, 187 cases with histologic follow-up were included. Rates of malignancy and the mutation profile of FNHCT were compared to 431 FN previously reported from our institution. Molecular testing using a 7-gene panel to detect mutations in *BRAF*, *K-*, *H-*, and *NRAS* genes and translocations involving *RET/PTC1*, *RET/PTC3* and *PAX8/PPARγ* was performed on indeterminate thyroid aspirates with results available for 172 FNHCT and 384 FN.

Results: Risk of malignancy for FNHCT was significantly lower than that for FN (21.4% vs 29.7%, p=0.039). The nature of malignant lesions also differed between these cytologic categories with follicular carcinomas more common on follow-up of FNHCT compared to FN (8% vs 1.4%). The rate of mutations was slightly higher in the FNHCT category (19.8% vs 16.7%, p=0.4). However, the pattern of mutation was somewhat different with FNHCT more commonly having *KRAS* mutations (6.4% vs 1.3%, p=0.002). *HRAS* mutations were more common in FN (1.2% vs 3.6%, p=0.168). The overall rate of *BRAF* mutations was similar (2.3% in FNHCT vs 1.8% in FN) but FNHCT more frequently had V600E mutations (1.7% vs 0.3%, p=0.09) and FN more frequently had K601E mutations (0.6% vs 1.6%, p=0.445). The risk of malignancy in molecular positive FNHCT was much lower than that of molecular positive FN (61.8% vs 93.8%, p=0.0001).

Conclusions: Risk of malignancy is lower in FNHCT compared to FN. This difference in risk of malignancy is more pronounced in molecular result positive cases although even in this group there is a fairly high risk of malignancy in the FNHCT molecular positive subset. These differences may be associated, in part with the higher rate of *KRAS* mutations in this category.

356 BRAF-V600E Detection From Stained Cytology Smears in Thyroid Carcinoma Fine Needle Aspirations Utilizing Clamp qPCR Technology

Laleh Hakima, Eli Flatow, Evan Pieri, Samer Khader, Antonio Cajigas, Mark Suhrland, Maja Oktay, Sumanta Goswami. Montefiore Medical Center, Bronx, NY; Yeshiva University, New York, NY.

Background: Mutational analyses are increasingly important for guiding treatment decisions. This is particularly important for surgical management of thyroid nodules with indeterminate cytological diagnoses. Thyroid lesions are frequently diagnosed using fine needle aspiration (FNA) cytology as they are highly vascular and a more aggressive method of tissue collection may result in hemorrhage. Molecular diagnostic tests of cytologic samples are commonly performed using paraffin embedded tissue (PET); however, insufficient cellularity presents an obstacle. Several studies reported successful molecular diagnostic tests performed from Diff Quik-stained cytology smears.

This approach ensures that material sent for molecular testing is representative of the lesion and is associated with a rapid turnaround time. We successfully determined the presence of the BRAF-V600E mutation in papillary thyroid carcinomas (PTC) and follicular thyroid carcinomas (FTC) using clamp qPCR technology on cytology smears. Clamp qPCR is highly sensitive detecting mutant DNA in as few as 50 cells with a 2% mutation detection level, compared to the reference lab threshold of 400 cells and 10% detection level.

Design: FNA smears from 24 cases of PTC and 2 cases of FTC with corresponding PET were collected. Areas of interest were marked on the Papanicolaou and Diff Quik-stained smears and DNA was extracted. BRAF-V600E mutant DNA was selectively amplified and detected using clamp qPCR method. Corresponding PET was tested in parallel to assess concordance. Two cases of PTC, one positive and one negative, were sent for reference laboratory testing which validated our method.

Results: Of 22PTC cases with unknown mutation status, 17 (11 classic, 5 follicular variant, 1 micropapillary) tested positive for BRAF-V600E and 5 (3 classic, 2 follicular variant) tested negative on both direct cytology smears and PET. Of two FTC cases, both tested negative on cytology smears and PET. There was 100% concordance between direct cytology smears and PET.

Conclusions: Molecular testing of FNA smears reliably detects DNA mutations in thyroid cancers and shows 100% concordance with PET. The percentage of BRAF-V600E (77%) in this study is higher than the percentage reported in literature (50%). These 2 observations suggest that molecular testing of cytology smears utilizing clamp qPCR technology has increased sensitivity to molecular testing using PET and may improve quality of patient care because FNA is minimally invasive and offers a quick turnaround time.

357 Cytology Specimen for Next-Generation Sequencing, Institutional Experience

Min Han, Jeff Wang. Washington University, St. Louis, MO.

Background: Next-generation sequencing (NGS) is a powerful technique for personalized medicine. Cytology specimens including cell blocks (CBs) and smears have been recently used for NGS but are faced with the challenge of inadequacy due to small quantities.

Design: We performed an institutional CoPath database search for all cytology materials and 85 consecutive small needle biopsies during 1-year period (2013-2014). Among 56 requests for NGS on in-house CBs and smears, 26 (25 CBs, 1 smear) passed the adequacy evaluation. 58 of 85 needle biopsies evaluated for NGS led to successful tests. We compared the success rate (SR) of cytology specimens for NGS with that of concurrent needle biopsies. In addition, we investigated the effect of dedicated pass numbers for CBs on the SR of NGS.

Results: The success rate of NGS on cytology specimens (46.4%) was lower than that of needle biopsies (68.2%) (P= 0.016, Chi square test). However, the findings of clinically actionable or prognostic variants in both groups were comparable: 10 of 26 (38.5%) cytology cases and 20 of 58 (34.5 %) needle biopsy cases. The successful NGS specimens were aspirated +/- ultrasound guidance from lymph nodes (LN), lungs, pancreas and body fluids .

Procedures	Bx sites	SR (total)
EBUS	Lung	54.5% (11)
	LN	52.2% (23)
EUS	Pancreas	100% (3♦)
	Liver	0 (4)
	LN	0 (2)
US	Lung	0 (2)
	LN	0 (3)
Superficial	LN	0 (1)
	Fluid	71.4% (7)

Note: EBUS - endobronchial ultrasound guided FNA; EUS - endoscopic ultrasound guided FNA; US - ultrasound guided FNA

♦2 of 3 cell blocks were suboptimal due to low DNA input

The average number of passes for CBs was 3.3 in adequate specimens and 2.8 in inadequate specimens (p=0.25, t test), with a range of 2-6 and 0-5 respectively. On-site evaluation by cytologists may improve the SR. All adequate cases had on-site evaluation and 5 inadequate cases didn't.

Table 2. Success rate (SR) of NGS by Number of Dedicated Passes for CBs

Passes (#)	SR	Num of cases
0	0	2
1	0	2
2	70.0%	10
3	17.6%	17
4	100.0%	3
5	42.9%	7
6	100.0	1
Unknown	50.0%	6

Conclusions: Cytology materials provide good alternatives for NGS with comparable clinically-relevant findings to needle biopsy specimens. FNA physicians' expertise, prioritization of specimens for NGS and cytologists on-site evaluation may improve the success rate for NGS. Two or more dedicated passes for CBs are recommended for a successful NGS test.

358 5-Year Cumulative Risk of CIN3 Following HPV Testing for ASC Cytology in Ages 21-24 in a Large Academic Center

Courtney Hanna, Janie Roberson, Allison Wrenn, Isam Eltoum. University of Alabama, Birmingham, AL.

Background: In recent ASCCP guidelines, risk of CIN3 and cancer in women 21-24 is considered low enough that the preferred management of ASCUS and LSIL is cytology follow-up rather than immediate colposcopy. This is largely based on data from Northern California Kaiser Permanente Medical Care Program which may not accurately reflect the pattern of squamous intraepithelial lesions (SIL) in other regions of the U.S. The objective of this study is to assess CIN3 cumulative risk (CR) following reflex HPV test (R-HPV-T) for ASCUS cytology in age group 21-24 yrs old compared to other screening age groups.

Design: In this is a retrospective study, results of R-HPV-T were correlated to subsequent outcomes. All women with a diagnosis of ASCUS and R-HPV-T in the period January 2000 through November 2011 were included. Cytology, histology, and Hybrid Capture 2 (HC2) R-HPV-T results were retrieved from Cerner System and re-entered into SPSS and XLSTAT for analysis. CR and confidence interval (CI) for HSIL or CIN3+ were calculated. CR and CI were stratified by age and HPV result. Results were significant at $p< 0.05$.

Results: 103,254 women (ages 13-95) were screened with 4,775 R-HPV-T performed. The mean (SD) numbers of pre- and post- R-HPV-T Pap tests were 2.1 (2.3) and 2.9 (2.0), respectively. The ASCUS-HPV+ rate was 37% (CI 36-38%); higher in those with a history of ASC+ (46%, CI 42%-49%), than those without that history (27%, CI 25%-29%). The CR(CI) for CIN3+ 5 years after positive R-HPV-T was higher than after a negative R-HPV-T in all age groups.

AGE GROUP	REFLEX-HPV TEST	RISK(%) of CIN3+ (95% CI)
	Negative	(0.0, 0-0)
	Positive	(2.6, 0.8-8.7)
21-24	Negative	(1.9, 0.6-6.2)
	Positive	(6.0, 3.4-10.8)
24-29	Negative	(0.4, 0.1-3.1)
	Positive	(11.5, 5.6-23.7)
>29	Negative	(1.0, 0.5-1.7)
	Positive	(5.4, 3.6-8.2)

The CR(CI) for CIN3+ five yrs after a positive R-HPV-T in age 21-24 was not significantly different than in age >29 yrs, but was significantly different than in age <21 yrs.

Conclusions: In our population, the risk of CIN3+ in women 21-24 yrs old is higher than the risk in age group <21 yrs, and is similar to that seen in the >29 age group. This finding is different than NCKPCP and may be explained by the prevalence of the disease or practice patterns, and calls for reassessing follow-up based on the prevalence of SIL and practice patterns.

359 Development of a Computer-Aided Decision Support Tool for Differentiating Between Cells Infected With Polyoma Virus and High Grade Urothelial Carcinoma in Urine

Jennifer Hipp, Dorothy Rosenthal, Ulysses Balis, Christopher VandenBussche. Johns Hopkins Hospital, Baltimore, MD; University of Michigan Health System, Ann Arbor, MI.

Background: Image analysis has created new opportunities for pathologists to perform quantitative assessments of cytologic features. One promising pattern matching algorithm, Spatially-Invariant Vector Quantization (SIVQ) has shown promising results for facilitating the detection of subtle architectural and nuclear features, making a compelling case for the exploration of its utility to differentiate between Polyoma virus (BK)-infected "decoy cells" and high grade urothelial carcinoma (HGUC).

Design: Images were obtained from Papanicolaou-stained liquid-based preparations of urine samples from 8 patients (4: decoy cells; 4 HGUC). Use of a region-of interest extraction tool, dCore, followed by use of an image aggregation tool, ImageMicroArray Maker, allowed for the generation of a montage of diverse examples of BK, HGUC, and benign urothelial cells.

Results: An initial vector set was utilized to differentiate atypical/suspicious cells from benign urothelial cells based on chromatin pattern. The second set of vectors was chosen for their ability to exhibit high affinity for a "ground glass" chromatin pattern and a dense nuclear rim seen in decoy cells. These vectors were orthogonal in their detection features; combining them identified four patients without falsely detecting HGUC cells (sensitivity 100%, specificity 100%).

Conclusions: Combined, multi-vector-based SIVQ detection is able to efficiently identify the nuclear features of decoy cells with a high sensitivity and specificity, while avoiding false-positive detection of HGUC cells and benign urothelial cells. This study provides pilot data demonstrating SIVQ as a suitable means to address the challenge of automated discerning cells suspicious for high grade urothelial carcinoma from decoy cells.

360 Usefulness of FISH Analysis Combined With Immunofluorescence in the Cytological Diagnosis of Mesothelioma in Serosal Effusions

Kenzo Hiroshima, Di Wu, Mizue Hasegawa, Yasuo Sekine, Daisuke Ozaki, Toshikazu Yusa, Yuji Tada, Hideaki Shimada, Masatoshi Tagawa. Tokyo Women's Medical University, Yachiyo, Japan; Chiba Rosai Hospital, Ichihara, Japan; Chiba University, Chiba, Japan; Toho University, Tokyo, Japan; Chiba Cancer Center Research Institute, Chiba, Japan.

Background: Mesotheliomas often present with serosal effusions, which are submitted for cytological evaluation. However, there is doubt as to the ability of the cytopathology to establish a definitive diagnosis of mesothelioma because of morphological overlap between malignant and benign mesothelial proliferation. There is no immunohistochemical staining that allows definite separation in this setting. Fluorescence in situ hybridization (FISH) of *p16* gene is helpful for this differentiation. However, there are few data on FISH analysis and no date on cutoff value for cell blocks.

Design: Eighteen cell blocks of serosal effusions with atypical mesothelial cells from 16 patients who had been histologically confirmed to have non-sarcomatoid pleural mesothelioma between 1995 and 2014 were stained with H&E and immunohistochemistry. The cell blocks and biopsy samples of the tumor of the same patients were analyzed with *p16* FISH and compared. FISH analysis on cell blocks was followed by immunofluorescence with epithelial membrane antigen (EMA).

Results: Clusters of atypical cells were observed in 83% (15/18) of the cell blocks. Eight cell blocks contained large clusters (^{3}100mm), seven contained small clusters (<100mm). Homozygous deletion (HD) was observed in 11 (69%) of biopsy samples of the pleural tumors. All cell blocks (12/12) from the patients with mesothelioma with HD harbored HD. Ten of 12 mesotheliomas with HD had HD pattern in more than 65% of analyzed cells in cell blocks. Five of six mesotheliomas without HD had HD pattern in less than 20%. Concordance of the results of FISH analysis between cell blocks and pleural tumor was good. Most of the clusters with HD were positive for EMA, but some were negative.

Conclusions: The presence of large clusters of atypical cells may be characteristic of mesothelioma. Immunofluorescence helps to identify the mesothelial cells in FISH analysis. Cytological diagnosis of mesothelioma with effusions could have been possible in two-thirds of the cases because atypical cells were confirmed to be mesothelial origin with immunohistochemistry and cell blocks harbored HD of *p16* gene. However, some cell blocks without HD had HD pattern in more than 20% of the analyzed cells. This may be due to poor fixation of tumor cells. We suggest that cutoff value for the diagnosis of mesothelioma with cell blocks should be higher than that for biopsy samples.

361 Cytomorphologic and Clinicoradiologic Analysis of Primary Non-Hematologic Central Nervous System Tumors With Positive Cerebrospinal Fluid

Cheng-Ying Ho, Christopher VandenBussche, Alison Huppmann, Rabia Chaudhry, Syed Ali. Children's National Medical Center, Washington, DC; Johns Hopkins Hospital, Baltimore, MD.

Background: Positive cerebrospinal fluid (CSF) cytology typically indicates leptomeningeal dissemination of metastatic, secondary or rarely, primary central nervous system (CNS) tumors. Large-scale studies on clinico-cytologic features of various primary CNS tumors in CSF are lacking.

Design: We performed a retrospective cytomorphologic study on 127 positive CSF specimens from 87 patients with a history of primary non-hematologic CNS tumor. Pertinent clinical, radiological and histologic findings were reviewed.

Results: Pediatric tumors accounted for the majority (82.6%) of the primary CNS tumors with positive CSF cytology. The most common radiological finding of neuraxial dissemination was diffuse leptomeningeal enhancement. More than 95% of the cases with positive CSF cytology were high-grade or malignant tumors. The most common tumor type was central primitive neuroectodermal tumors (cPNETs) (47.2%). Overall, the frequency of initial metastasis was the lowest in cPNETs and retinoblastomas (~ 2/3). They also had the longest latency (1.5-2 years) in cases without initial metastasis. Most metastatic tumors in CSF demonstrated distinct cytomorphology reminiscent of the histologic features of the primary tumor, such as prominent nucleoli, cell wrapping and apoptosis in large cell/anaplastic medulloblastomas, rhabdoid morphology and cytoplasmic inclusions in atypical teratoid/rhabdoid tumors, large clusters of cells with scant cytoplasm and nuclear molding in retinoblastoma, nuclear pleomorphism and hyperchromasia in high-grade infiltrating astrocytomas, and small clusters/rosettes of epithelioid cells in ependymomas.

Conclusions: Our study provides useful clinico-radiological information and cytomorphologic illustrations for both common and rare primary CNS tumors that cytopathologists might encounter on CSF examination.

362 Diagnostic Yield in Cytopathological Evaluation of Pericardial Effusions – Clinicopathological Analysis of 472 Specimens

Rana Hoda, Jad Saab, Brian Geraghty, Syed Hoda, Jeffrey Port, Tamara Giorgadze. New York Presbyterian Hospital – Weill Cornell Medical Center, New York, NY.

Background: Pericardial effusion (PE), regardless of etiology, causes considerable morbidity and contributes to mortality. Malignant PE are considered uncommon, and data on malignancies encountered in cytopathological examination of pericardial effusion (CEPE) are scant (*Dragoescu.Cancer Cytopathol*. 2013;121:242-251).

Design: Relevant records of all specimens submitted for CEPE, over an 80-month period (01/2008-09/2014) were examined. Clinical history and findings of CEPE and synchronous/metachronous pericardial/anterior mediastinal (i.e. regional) surgical pathology biopsy (SPB) were tabulated.

Results: 4988 (pleural, peritoneal and pericardial) effusion specimens were received for cytopathological evaluation during the study period. 472 (9.4%) of these were PE specimens—obtained from 364 patients (men: 176, women: 188; age range: 2-95; mean, 63). 377 of 472 (80%) PE specimens were "negative", 21 (4%) were "atypical", 8 (2%) were "suspicious", and 66 (14%, from 51 patients) were "positive" for malignant cells i.e. (+)CEPE. 46 of 51 (90%) patients were known to have malignancy. 11 (of 51, 22%) patients with (+)CEPE did not show metastases elsewhere. The most common primaries were in lung (20/51, 39.2%), breast (11/51, 21.6%) and gastrointestinal tract (7/51, 13.7%). 5/51 (9.8%)patients had unknown primary. The most common primary in women and men was breast (11/31, 35.5%) and lung (9/20, 45%), respectively. 96 of 364 (26%) patients presented with pericardial tamponade, of which 27 (28%) had (+)CEPE. Regional SPB was performed in 288 of 364 (79%) patients. (+)CEPE correlated with concurrent (+)SPB in 241/288 (83.7%). 24/288 (8.3%) had (+)CEPE with concurrent (-)SPB. 23/288 (8.0%) had (-)CEPE with (+)SPB. All discrepancies were seemingly attributable to sampling, rather than interpretative, issues.

Conclusions: In this series of CEPE, (i) Specific cytological diagnosis of "negative" or "positive" was rendered in 94% of cases; (ii) 14% of specimens were positive for malignancy; (iii) breast and lung were the most common primary in women and men, respectively; (iv) 11 of 51 (22%) patients with (+)CEPE showed no evidence of metastases elsewhere; (v) Discrepancy rate of CEPE and SPB was similar (8%); (vi) (+)CEPE as the initial manifestation of malignancy was only seen in 5 (10%) patients as majority, 90%, had a history of malignancy.

363 Performance of the Afirma Gene Expression Classifier in Hurthle Cell Thyroid Nodules

Brittany Holmes, Eran Brauner, Jeffrey Krane, Sareh Parangi, William Faquin. Massachusetts General Hospital, Boston, MA; Brigham & Women's Hospital, Boston, MA.

Background: The recently introduced Afirma gene expression classifier provides binary results (suspicious/benign) to guide clinicians in the setting of indeterminate fine needle aspiration (FNA) cytology. Afirma testing is intended to reduce unnecessary surgeries for benign nodules, and management algorithms favor thyroidectomy for suspicious results. Little data is available on the characteristics of this test for Hurthle cell nodules, which are otherwise managed by lobectomy. We hypothesized that a predominance of Hurthle cells leads to an increased rate of suspicious Afirma results with a potential for overtreatment, despite a relatively low risk of malignancy.

Design: A multi-institutional pathology database was queried from 2010 to 2014 for all FNAs diagnosed as suspicious for Hurthle cell neoplasm (SHCN) or atypia of undetermined significance/follicular lesion of undetermined significance with a predominance of Hurthle cells concerning for an oncocytic neoplasm (AFHCN). Cytology diagnoses were rendered internally prior to Afirma testing. The patient demographics, FNA diagnosis, Afirma result, surgical procedure, and pathologic outcome were recorded. Out of 234 thyroid FNAs diagnosed as SHCN or AFHCN from 199 unique patients, 45 were sent for Afirma testing, including 26 SHCN aspirates and 19 AFHCN aspirates.

Results: Of 45 cases tested, the Afirma result was suspicious in 78% (n=35, including 23 SHCN and 12 AFHCN). Twenty-eight of these patients underwent surgical resection (20 SHCN and 8 AFHCN), and four (14%) showed malignant final pathology, specifically two oncocytic papillary thyroid carcinomas (PTC) and two Hurthle cell carcinomas (HCC). In addition, three patients with benign Afirma results underwent resection, all of which yielded benign pathology. Among cases not sent for Afirma testing, the baseline rate of malignancy in nodules diagnosed as SHCN was 26% (13% PTC, 13% HCC).

Conclusions: In the setting of an FNA with a predominance of Hurthle cells, the positive predictive value of a suspicious Afirma result is 14%. Given the pre-test malignancy risk of 15-30% for an FNA diagnosis of suspicious for follicular neoplasm, a suspicious Afirma result does not increase the probability of malignancy in a Hurthle cell nodule. Due to the low risk of PTC in these patients, lobectomy without Afirma testing may represent an appropriate management algorithm.

364 Rapid On-Site Evaluation: A Comparison of Performance of Pulmonologist To Cytopathologist

Susanne Jeffus, Nikhil Meena, Nicole Massoll, Chien Chen, Soheila Korourian, Thaddeus Bartter. University of Arkansas for Medical Sciences, Little Rock, AR.

Background: At our institution, rapid on-site evaluation (ROSE) is routinely provided by cytopathologists. ROSE has been shown to increase diagnostic yield, aid in specimen triage for ancillary tests and decrease procedure time. However, lack of time, personnel, or cost can preclude cytopathologists from providing a ROSE service or from consistently attending procedures. Proceduralist participation could benefit both parties. This study evaluated the capacity of pulmonologists to determine on-site adequacy (OSA) and assign a diagnostic category and the impact of pulmonologist pre-screening of slides upon cytopathologist time commitment.

Design: Interventional pulmonologists were given educational training on cytomorphology and OSA. Diff-Quik-stained smears were evaluated first by a pulmonologist and subsequently by a cytopathologist, each party blinded to the other's ROSE. For each case, site, number of passes, OSA, diagnostic category (non-diagnostic, benign, atypical, suspect for malignancy, malignant) and cytopathologist ROSE time were recorded. Pulmonology and cytopathology (gold standard) performance was compared.

Results: 154 sites (lymph nodes: 125, lung mass: 20, other sites: 9) from 109 patients were sampled with 3.7 ±1.7 passes per site. There was near perfect agreement between observers for OSA {0.88 (0.83 to 0.93), p-value < 0.001}. Agreement for diagnostic categories is shown in Table 1.

Diagnostic Category	Cytopathology Observations (n)	Pulmonology Agreement (%)	K(95% CI)	p value
Overall Agreement	154	91	0.82(0.76-0.888)	
Non-Diagnostic	3	100	1.00 (1.00-1.00)	
Benign	72	93	0.87(0.795-0.954)	
Atypical	15	45	-0.082(-0.4942-0.328)	0.24
Suspect for Malignancy	3	33	0.00(-0.49-0.49)	0.34
Malignant	61	97	0.94(0.89-1.00)	

Time spent by cytopathologists in the endoscopy suite averaged 4.02 ± 6.9 minutes per procedure (range 1 to 21 minutes) compared to traditional 20 ± 10 min.
Conclusions: 1. Trained pulmonologists can ensure OSA, which may be of value in the absence of a pathology provided ROSE service. 2. The ability of pulmonologist pre-screening of slides leads to dramatic time economy for the cytopathologist in the setting of a very busy ROSE service. 3. ROSE can be optimized as a collaborative effort between pulmonologist and cytopathologist.

365 Utility of Fine Needle Aspiration and Core Needle Biopsy in the Diagnosis and Classification of Lymphoma: A Single Institution Experience

Fatima Zahra Jelloul, Maria Navarro, Pooja Navale, Tamla Hagan, Xinmin Zhang, Silvat Sheikh-Fayyaz. North Shore-LIJ Hofstra School of Medicine, New Hyde Park, NY.
Background: For many years, excisional biopsy has been the gold standard method for the diagnosis and classification of lymphoma. However, there has been an increasing use of fine needle aspiration (FNA) with and without core needle biopsy (CNB) due to ease of procedure and less complications as compared to excisional biopsy. We sought to evaluate our experience with FNA with and without CNB and available ancillary studies in diagnosing and subclassifying lymphoma according to the 2008 World Health Organization (WHO) classification.
Design: A search was conducted in our information system for patients who underwent FNA with or without CNB of any site for a suspected diagnosis of lymphoma, for a period of two years (2012 and 2013). We identified 338 cases (290 from lymph nodes and 48 from extranodal sites). Ancillary studies such as flow cytometry, immunohistochemical stains and molecular tests were also reviewed.
Results: Overall results included 122/338 (36.1%) positive, 24/338 (7.1%) suspicious, 25/338 (7.4%) atypical and 167/338 (49.4%) negative for diagnosis of lymphoma. Of the 122 positive cases, 77 had FNA with CNB and 45 had FNA with no CNB. A complete subclassification according to WHO was made in 94/122 (77.1%) of positive cases. There was a significant difference in subclassification between the cases that had FNA with CNB and the cases that had FNA with no CNB (chi-square, P<0.0001). Specifically, 71/77 (92.2%) of cases having FNA with CNB were subclassified, whereas only 23/45 (51.1%) of cases with FNA and no CNB were subclassified. Subclassification of all 94 cases is reported in table 1.

Type of Lymphoma	Number of Positive cases	Percentage (%)
Diffuse Large B Cell (DLBCL)	29	30.9
Follicular	23	24.5
CLL/SLL	15	16.0
Hodgkin	8	8.5
Plasma Cell Neoplasm	4	4.3
Myeloid Sarcoma	4	4.3
Marginal Zone	3	3.2
Mantle	3	3.2
B Lymphoblastic	2	2.1
DLBCL, EBV related	1	1.0
T Lymphoblastic	1	1.0
Burkitt	1	1.0

Conclusions: Our study shows a high diagnostic accuracy with combined FNA and CNB along with ancillary studies in the diagnosis and subclassification of lymphoma. It is a cost effective and reliable method that is becoming the standard procedure for evaluation of lymphoproliferative disorders.

366 What Is the Positive Predictive Value (PPV) of the Diagnosis ldquo] Suspicious for High Grade Urothelial Carcinoma (SHGUC)" in Urinary Tract Cytology (UTCy)?

Anthony Joudi, Stefan Pambuccian, Eva Wojcik, Guliz Barkan. Loyola University Medical Center, Maywood, IL.
Background: In an effort to standardize the practice of UTCy, a new reporting system (The Paris System, TPS) has been proposed, containing new diagnostic categories, including SHGUC. However, the PPV associated with this reporting category of SHUC is currently unknown. Since in our institution SHGUC has been diagnosed for over 10 years, we have reviewed our experience with SHGUC to determine the PPV associated with this diagnosis.
Design: Cases diagnosed as SHGUC and positive for high grade urothelial carcinoma (PHGUC) from 01/01/2003 to 12/31/13 on urinary cytology which had any surgical biopsy, cytology performed within 6 months following the index test were included in the study. During the study period our institution processed 17,770 urine cytologies with 2.75% diagnosed as PHGUC and 1.00% diagnosed as SHGUC.
Results: We identified665 cases (178 SHGUC, 487 PHGUC), corresponding to 389 unique patients, 76% men with a mean age of 73.4 and 24% women with a mean age of 74.3. 149 had follow-up via histology only, 52 via cytology only, and 464 had both surgical and cytologic follow-up. Cases were considered positive for malignancy if at least one follow-up method resulted in a positive diagnosis [Table 1].

POSITIVE UTCy					SUSPICIOUS UTCy			
F/U	History of UC	Hema-turia	Other in-dications	Total	History of UC	Hema-turia	Other indica-tions	Total
Pos.	414	8	2	424	76	17	6	99
Neg (Incl. equivocal)	62	1	0	63	49	22	8	79
Total	476	9	2	487	125	39	14	178
PPV	86.97%	88.89%	100.00%	87.06%	60.80%	43.59%	42.86%	55.62%

Conclusions: The PPV of PHGUC (87.1%) was much higher than that of SHGUC (55.6%) (p<0.0001). The PPV of PHGUC did not vary according to the indication for urinary tract cytology (p=1); while the PPV of SHGUC appeared higher in patients followed for UC, but the difference was not statistically significant (p=0.19).

367 Endoscopic Ultrasound (EUS)-Guided Fine Needle Aspiration (FNA) Cytology of Gastrointestinal Stromal Tumors: A 21-Year Retrospective Study of 172 Cases

Stephen Jovonovich, Howard Wu, Shaoxiong Chen, Harvey Cramer. Indiana University School of Medicine, Indianapolis, IN.
Background: Gastrointestinal stromal tumors (GISTs) are the most common mesenchymal tumors of the gastrointestinal tract. Due to their deep location within the GI tract, they are difficult to diagnose by conventional surgical biopsy techniques. EUS-guided FNA has been increasingly used for the preoperative diagnosis of GISTs. In reviewing our experience, in this study, our aim is to determine the accuracy of EUS-guided FNA for the diagnosis of GIST.
Design: A computerized search of the cytopathology laboratory information system was performed and all cases in which a diagnosis of GIST was rendered or suggested by FNA were identified. All correlating surgical pathology report diagnosis were obtained and retrospectively reviewed.
Results: Over a 21-year period, a total of 172 EUS-guided FNA cases were diagnosed as GIST (140 cases, 81.4%), suggestive of GIST (14 cases, 8.1%), or as a spindle cell neoplasm in which GIST was in the cytologic differential diagnosis (18 cases, 10.5%). The patients included 90 males and 85 females with a mean age of 64 and an age range of 21 to 93 years. Gastric GISTs accounted for 128 of the 172 cases (74%). The tumors ranged in size from 0.6 to 17 cm (mean: 5 cm). The cytomorphologic diagnosis of GIST was supported by positive c-kit immunostaining performed on cell block sections in 56 patients. Surgical resection histologic follow-up was available for 107 patients. Follow-up histologic diagnoses other than GIST were found in 9 cases (8%): leiomyosarcoma (1 case), leiomyoma (3 cases), synovial sarcoma (1 case), plexiform schwannoma (1 case) and no pathologic change (3 cases). In all 9 of these cases confirmatory immunostaining could not be performed due to hypocellularity of the cell block. In 3 cases with no pathologic change on histologic follow-up, fragments of normal smooth muscle from the gastrointestinal wall might have been mistaken as lesional tissue.
Conclusions: EUS-guided FNA, along with confirmatory c-kit immunostaining, is an accurate method of establishing a preoperative pathologic diagnosis of GIST. In our study, subclassification errors generally occurred in cases in which confirmatory immunostaining could not be performed due to hypocellularity of the sample. Contamination from the gastrointestinal wall may also contribute to false positive diagnosis.

368 Cytologic Features in Metaplastic Bladder Conditions – Another Entity Associated With Atypia in Urine Cytology

Ruba Khattab, Hui Zhu, Jordan Reynolds. Cleveland Clinic Foundation, Cleveland, OH.
Background: Atypical transitional cells (ATC) represent a diagnostic challenge. These may be found in benign/metaplastic bladder conditions. We reviewed the morphologic features of urine cytology that are most frequently seen in follicular cystitis (FC), cystitis cystica (CC), cystitis cystica et glandularis(CCG) and papillary cystitis (PC). FISH results were also reviewed on a subset of cases.
Design: Medical records were searched for bladder biopsies over a 2 year period (January 2012 to December 2013) with a diagnosis of FC, CC, CCG or PC, without history of genitourinary malignancy. Cases with corresponding urine cytology within a six month period were reviewed for the following features: cellularity, background, architecture, and nuclear features. Histologic slides were reviewed. The histologic type of cystitis, extent and severity of involvement were recorded. Interphase FISH using Urovysion kit was reviewed and recorded on the cases on which testing was performed.
Results: 69 patients were diagnosed with FF, CC, CCG or PC on bladder biopsy with no history of urothelial carcinoma that had corresponding urine cytology (n=98 cytology cases). Interphase FISH was performed on 14 cytology cases. The cytologic diagnoses were negative for malignancy (72/98) ATC (25/98) and positive for malignancy(1/98). FISH was negative in 11/14 cases, positive in one (1/14) case, nondiagnostic in one case (1/14), and insufficient for diagnosis in one case(n=1).

The most pertinent finding was that of signet ring cells and glandular cells with vacuolated cytoplasm (n=13 13.2%) (Table 1). These cells were characterized by an eccentrically located nuclei, inconspicuous nucleoli and vacuolated cytoplasm.

Case	Cytology	Histology
1	ATC	CCG
2	Negative	CCG
3	Negative	CC
4	Negative	CC
5	ATC	PC with focal CC
6	ATC	PC with focal CC
7	Negative	CC
8	ATC	CC and FC
9	Atypical squamous cells	PC and CC
10	ATC	CC and FC
11	Rare Atypical cells with degenerative changes	CC,radiation cystitis
12	Negative ,polyoma changes	CCG
13	Negative	CCG

Conclusions: Benign metaplastic bladder conditions may exhibit ATCs, representing a diagnostic pitfall. The most common findings in these specimens are signet ring like and columnar cells (13% of cases). Signet ring like cells in benign metaplatic bladder conditions represent another entity mimicking ATC. Interphase FISH performed on such cases show a high negative predictive value.

369 Abdominal Fat Pad Aspirate in Systemic Amyloidosis

Fumiko Konno, Chiraag Patel, Ahmet Dogan, Oscar Lin. Memorial Sloan Kettering Cancer Center, New York, NY.

Background: Systemic amyloidosis is a multiorgan disease and is associated with significant morbidity and mortality. The diagnosis depends on identifying amyloid in tissues. Microscopic examination of abdominal fat pad fine needle aspiration (FNA), stained with Congo Red and/or evaluated by electron microscopy studies, is a widely used technique for the diagnosis of systemic amyloidosis. However, the reported sensitivity and specificity of Congo Red is highly variable, especially in early stage amyloidosis. Electron microscopy (EM), on the other hand, is frequently used as a confirmatory test due to its high reported specificity; however it is offered in only small number of institutions. This study is a large, single institution study of the performance of Congo Red stain in the diagnosis of systemic amyloidosis.

Design: Our pathology database was queried for abdominal fat pad FNA specimens, stained with Congo Red, obtained during 2009 to 2013. The slides were retrieved and examined microscopically, under polarized light, for the presence of apple-green birefringence. These results were compared to the corresponding EM results. Furthermore, these results were correlated to the patient's clinical history.

Results: We identified 185 cases of abdominal fat pad FNA stained with Congo Red stain which had concurrent EM results. Of the 185 cases, 162 (88%) cases had concordant Congo Red and EM results. Among these 162 cases, 33 cases had concordant positive results while 129 cases had concordant negative results. The kappa value between both tests was high (0.82), which was significant ($p<0.1$). The concordance was remarkable in patients who clinically had no evidence of systemic amyloidosis, however, the kappa value was lower (0.6) in the cohort of patients with clinical diagnosis of systemic amyloidosis. There were 5 cases where Congo Red had a positive result while EM was negative. In 4 cases, only EM was positive.

Conclusions: Congo Red results correlates well with EM results, particularly in patients without clinical suspicion of systemic amyloidosis. Although EM was found to be more sensitive and specific in previous reports, we found 5 false negative cases of EM. Our study demonstrates that Congo Red and EM can be used interchangeably in most cases, especially when the clinical suspicion is low. Documentation of amyloid deposition in a patient highly suspected to have systemic amyloidosis might benefit from the use of both Congo Red and EM.

370 Drastic Loss of MIB1/Ki67 Immunoreactivity in CytoLyt-Fixed Cell Blocks

Fumiko Konno, Achim Jungbluth, Denise Frosina, Miriam Fayad, Marcia Edelweiss, Oscar Lin, Natasha Rekhtman. Memorial Sloan Kettering Cancer Center, New York, NY.

Background: Quantitative assessment of MIB1 (Ki67) proliferation marker immunoreactivity is a well-established and widely-utilized ancillary tool for tumor diagnosis and prognostication. In our cytopathology practice, we have encountered a number of cases with unexpectedly low MIB1 immunoreactivity in cell blocks (CBs). This study was designed to study the impact on MIB1 immunoreactivity of CytoLyt (methanol-based medium) vs formalin fixation as well as other CB processing and immunocytochemical procedures.

Design: Freshly resected carcinomas of various sites (n=6) were gently scraped at the time of specimen prosection to prepare test CBs. Each scrape was divided evenly into two containers - CytoLyt or 10% buffered formalin. Each container was split in half and processed by (1) standard method in our cytology laboratory, employing Histogel and Ethanol (EtOH) to dehydrate and concentrate the sample; and (2) same method but excluding EtOH step. Immunocytochemistry for Ki67 (MIB1; DAKO; 1:200) was performed on Ventana XT and Leica-Bond-3. The percentage of tumor cells labeling with MIB1 was assessed semiquantitatively.

Results: MIB1 immunoreactivity in standard CytoLyt-fixed CBs on Ventana XT platform from 6 tumors was 0%, 10%, 0%, 0%, 10%, and 3%, whereas MIB1 immunoreactivity in corresponding formalin-fixed CBs (no EtOH step) was 25%, 90%, 30%, 95%, 95%, and 85%, respectively. This represents the average loss of MIB1 immunoreactivity of 66% (range 25-95%) in CytoLyt compared to formalin-fixed material. EtOH step resulted in variable decrease in MIB1 immunoreactivity in formalin-fixed CBs (average decrease 8%, range 0-30%), and further decrease in CytoLyt-fixed CBs (average decrease 8%, range 0-25%). Results on Leica-Bond-3 platform were comparable to those on Ventana XT. Modification of antigen retrieval and/or antibody concentration did not recover MIB1 immunoreactivity in CytoLyt-fixed CBs.

Conclusions: Here we document that fixation of cytological specimen in CytoLyt leads to a major inhibition of MIB1 immunoreactivity, which in some cases is further exacerbated by addition of EtOH step during CB processing.We are not aware of prior publications describing this issue. This issue may present a treacherous diagnostic pitfall because some tumor classifications rely on quantitative assessment of MIB1, which would be markedly discrepant in CytoLyt-fixed CBs. We are in the process of evaluating other anti-Ki67 antibodies in CytoLyt vs formalin-fixed CBs.

371 Interobserver Reproducibility of Cytomorphologic Features Distinguishing Sarcoidosis From Infection By FNA

Michael Kyle, Matthew Keeney, Thomas Wilson, Michael Gailey, Chris Jensen. University of Iowa, Iowa City, IA.

Background: Sarcoidosis and infection (esp. fungus, Mycobacteria) are two of the most common causes of granulomas in pulmonary and mediastinal fine needle aspiration (FNA). Although these processes can be difficult to distinguish on morphologic features, several recent studies have suggested that cytomorphologic features, including the number of granulomas, may help distinguish between the two at FNA. However, it is unclear if these features can be reproducibly recognized by multiple observers with varying levels of experience. This study was designed to measure the interobserver reproducibility of several cytomorphologic criteria.

Design: 40 cases of histoplasmosis and sarcoidosis (20 each), without extensive necrosis, in which the diagnosis was made by clinicopathologic correlation were examined by four reviewers blinded to the final diagnosis. Presence/absence of necrosis, quantity of granulomas (few, moderate, many), character of granulomas (angular vs round), presence of giant cell infiltrate, presence of tissue fragments, and presence of acute and chronic inflammation were assessed. Concordance was estimated using the intraclass correlation coefficient.

Results: See Table 1.

	Intraclass correlation coefficient (ICC)	Lower limit confidence	Upper limit confidence
Presence of acute inflammation	0.73	0.61	0.83
Quantity of granulomas	0.66	0.48	0.79
Presence of necrosis	0.64	0.49	0.76
Presence of giant cells	0.52	0.36	0.68
Presence of tissue fragments	0.49	0.33	0.65
Character of granulomas	0.45	0.27	0.62

Conclusions: 1. Observers demonstrated modest reproducibility for the presence of acute inflammation and necrosis along with a semiquantitative assessment of granuloma number with ICC of 0.73, 0.66 and 0.64, respectively. This is reassuring as these were the most important criteria identified in prior studies for distinction of sarcoidosis from infection.

2. Non-quantitative characteristics of granulomas including character (round vs angular), presence of solid tissue fragments and presence of giant cells were less reproducible (ICCs < 0.5 for most). These less reproducible characteristics have been shown previously to be less valuable for discriminating the etiology of granulomas.

3. Observer experience did not appear to impact correlation, as ICCs did not improve significantly when less experienced observers were removed from the comparison.

4. Training sets and correlation conferences have been shown to improve interobserver correlation and may be helpful with these criteria.

372 Interobserver Reproducibility and Agreement With Original Diagnoses for the Categories Atypical and Suspicious for Malignancy in Bile and Pancreatic Duct Brushings

Lester Layfield, Barbara Chadwick, Magda Esebua, Robert Schmidt, Benjamin Witt. University of Missouri, Columbia, MO; University of Utah, Salt Lake City, UT.

Background: The Papanicolaou Society of Cytopathology has developed a diagnostic scheme which includes the categories atypical and suspicious for malignancy. These intermediate categories help stratify risk of malignancy for samples but the reproducibility of these categories is unknown.

Design: Twenty sequential brushing specimens from each of the categories atypical and suspicious for malignancy were identified and the slides retrieved. All forty cases were reviewed independently by four cytopathologists blinded to the original diagnoses. Resulting review diagnoses were statistically analyzed and the Kappa statistic calculated. Agreement of observers with original diagnosis was also evaluated.

Results: Interobserver agreement was graded as slight to fair with observers agreeing about 50% of the time. The corresponding Kappa statistic for the category atypical was 0.21 and 0.18 for the category suspicious for malignancy. Reviewer agreement with the reference diagnosis occurred in about half of review diagnoses.

Conclusions: Kappa analysis shows that interobserver agreement is only slight to fair. Observers were able to reproduce the reference diagnosis of atypical or suspicious in only about half of review diagnoses. Despite the categories atypical and suspicious for malignancy having distinct risks of malignancy (62% versus 74%), the reproducibility of these categories is relatively poor. A single intermediate category may improve reproducibility over a four-tier system, while maintaining ability to stratify risk of malignancy.

373 Malignancy Risk Associated With EUS-FNA Diagnostic Categories "Non Diagnostic", "Benign", "Atypical", "Suspicious for Malignancy" and "Malignant" for Lymph Nodes

Lester Layfield, Magda Esebua, Benjamin Witt. University of Missouri, Columbia, MO; University of Utah, Salt Lake City, UT.

Background: Endoscopic bronchial ultrasound guided fine needle aspiration (EBUS-FNA) is frequently utilized for pre-operative staging of known or suspected primary carcinoma of the lung. The procedure is safe and is associated with acceptable diagnostic accuracy. Published diagnostic sensitivity and specificity are up to 86% and 100% respectively. As with EBUS-FNA of primary pulmonary lesions, diagnostic categories utilized by many cytopathologists are non-diagnostic, benign, atypical, suspicious for malignancy and malignant. Little information is available as to the risk of malignancy associated with each of these categories.

Design: The records of the Department of Pathology at the University of Utah/ARUP Laboratories were searched for all EBUS-FNA reports of mediastinal and pulmonary hilar lymph nodes. Only cases with surgical follow-up were included in this study. For each diagnostic category (non-diagnostic, benign, atypical, suspicious for malignancy and malignant) the percentage of cases surgically proven to be malignant was calculated following correlation of cytologic and surgical diagnoses. Additionally, diagnostic sensitivity and specificity were calculated. For statistical purposes, atypical cases were considered benign and suspicious for malignancy cases were classified as malignant.

Results: One-hundred and sixty-three EBUS-FNAs of pulmonary or mediastinal lymph nodes were obtained with adequate surgical follow-up. Risk of malignancy for non-diagnostic specimens was 42%, benign specimens 32%, atypical specimens 40%, suspicious for malignancy specimens 83% and malignant specimens 84%. Diagnostic sensitivity was 60% and diagnostic specificity was 88%.

Conclusions: The proposed cytologic categories stratified malignancy risk ranging from a low of 32% for benign aspirates to 84% for aspirates designated as malignant. The categories suspicious for malignancy and malignant had essentially the same malignancy risk. Aspirates designated atypical had a slightly higher malignancy risk than those designated benign. Non-diagnostic aspirates had a malignancy risk similar to those designated atypical. This categorization scheme with associated malignancy risks may be helpful for management decisions concerning staging of lung carcinomas.

374 Relationship Between Frequency of False Negative Diagnoses and Lymph Node Size and Percentage Nodal Replacement By Metastases in Axillary Lymph Nodes Undergoing FNA in Breast Cancer Patients

Lester Layfield, D Eric Ewing. University of Missouri, Columbia, MO.

Background: Ultrasound guided fine needle aspiration (FNA) is frequently utilized to sample sentinel lymph nodes in breast cancer patients. Diagnostic sensitivity and specificity have been reported as good but little data exists regarding causes for false negative results.

Design: Fifty-four sequential ultrasound guided FNAs of sentinel lymph nodes were identified which had surgical follow-up. In each case, size of lymph node and percentage of lymph node replacement by cancer were determined on Hematoxylin and Eosin stained sections of surgical specimens. Correlation was made between frequency of false negative results and lymph node size and percentage of lymph node replaced by tumor. Diagnostic sensitivity and specificity were calculated.

Results: Seventeen cases were positive by FNA of which three were false positives (3/54[6%]). Thirty-seven were cytologically negative with 5 being false negatives (9%). True positive lymph nodes averaged 1.9cm (range 0.25 to 2.4 cm) in greatest dimension while falsely negative nodes averaged 0.92cm (range 0.3 to 1.3cm) in greatest dimension. Percentage involvement for true positive FNAs averaged 69% (range 1 to 100%) while false negative cases averaged 25% (range 1 to 90%). Diagnostic sensitivity was 74% and specificity was 91%.

Conclusions: There exists a relationship between sentinel lymph node size and likelihood of a false negative result. Lymph nodes under 1.2cm in size have a higher incidence of false negative results than larger lymph nodes. A similar relationship exists with percentage node involvement by carcinoma with nodes having less than 30% involvement demonstrating a higher percentage of false negative results than nodes showing greater than 30% replacement by carcinoma. Sentinel lymph nodes under 1cm in maximum size appear to be relatively poor candidates for ultrasound directed FNA.

375 Post Hoc Assessment of B-Cell and T-Cell Gene Rearrangements From Cytology Smears

Thomas Lee, Ryan Phan, Phillipp Nham, Neda Moatamed. David Geffen School of Medicine at UCLA, Los Angeles, CA; VA Greater Los Angeles Healthcare System, Los Angeles, CA.

Background: The definitive diagnosis of lymphoma in cytology specimens can be challenging by cytomorphologic examination alone and requires further testing and confirmation by flow cytometry or molecular studies. However, limited or unavailable specimens may preclude additional testing. In this study, a novel approach of assessing cells retrieved from previously processed cytology slides was utilized for the first time to identify clonal immunoglobulin (Ig) and T-cell receptor (TCR) gene rearrangements.

Design: Nineteen fine needle aspiration (FNA) cases with concurrent surgical pathology and lymphoma work up diagnoses of lymphoproliferative disorder were selected for analysis. FNA smear slides from these cases were digitally scanned and prepared for cell recovery. Cells scrapped from the slides were then spun down into pellets for genomic DNA extraction. After testing the extracted DNA for quantity and quality, the results of PCR-based molecular analysis for Ig (IgH, Igκ, and Igλ) and TCR (TCR-β, TCR-γ, TCR-Δ) gene rearrangements were correlated with case diagnoses.

Results: Thirteen (68.4%) cases showed post hoc recovery of adequate DNA for PCR-based testing. Insufficient DNA was recovered in six (31.6%) cases. No statistically significant associations were seen between DNA adequacy and case diagnosis or smear preparation. The thirteen cases with adequate DNA consisted of three B-cell lymphomas, two T-cell lymphomas, and eight reactive lymphadenopathies. Clonal Ig and TCR gene rearrangements were seen in the three B-cell lymphoma and two T-cell lymphoma cases, respectively. One reactive lymphadenopathy case showed clonal IgH and Igk gene rearrangements. No clonal gene rearrangements were found in the remaining seven reactive lymphadenopathy cases. Overall, the post hoc recovery methodology demonstrated high concordance (92.3%) with case diagnoses in cytology specimens with adequate DNA.

Conclusions: The assessment of clonal gene rearrangements in lymphoproliferative disorders was demonstrated for the first time using cells recovered post hoc from processed cytology slides. This approach may be particularly useful when insufficient material is available for concurrent or follow-up flow cytometry and molecular studies. These results further support the prospect of rendering accurate diagnoses of lymphoproliferative disorders by FNA.

376 Comparison of Liquid Based Cytology of Direct Brush and Saliva Specimens in Oral and Oropharyngeal Squamous Cell Carcinomas

Marino Leon, Barbara Centeno, Elizabeth Kostas-Polston. H. Lee Moffitt Cancer Center & Research Institute, Tampa, FL; Saint Louis University School of Medicine, St. Louis, MO.

Background: From 1997 to 2007, the United States has experienced a 225% increase in oropharyngeal carcinomas (OPC). Human papilloma virus (HPV) infection is now recognized as a main etiologic factor of OPC in the US. The purpose of this study was to explore the relationship between exfoliative cytology and the histopathological findings in OPC and oral cavity carcinomas (OCC).

Design: Forty patients who presented to a Head & Neck Clinic with undiagnosed oral (n=20) and oropharyngeal (n=20) suspicious lesions were studied in an IRB approved protocol. A total of 40 direct brushing specimens (DBS) and 39 saliva samples were collected. The DBS cells were collected using an endocervical brush and placing the material in a PreservCyt solution (Hologic, Bedford, MA). Five mL of saliva from each patient were collected and preserved also in PreservCyt solution. All samples were routinely processed on a ThinPrep 2000 processor (Hologic). The slides were stained with Papanicolaou stain and examined by two cytopathologists. The specimens were classified as Diagnostic for Malignancy, Suspicious for Malignancy, Atypical Squamous Cells of Undetermined Significance (ASCUS), Negative for Intraepithelial Lesion or Malignancy (NILM), and Insufficient for evaluation. The cytological diagnoses were compared with the corresponding histological diagnoses. HPV status was determined using Cervista HPV HR assay (Hologic) on the DBS.

Results: The mean age was 58.4 years. Thirty-three males and 7 females were encountered. All patients were diagnosed with squamous cell carcinoma (SCC). HPV was found in 65% of OPC and 20% of OCC DBS. The cytological evaluation of the DBS and Saliva are presented in Table 1.

Cytopathologic classification	OPC		OCC	
	DBS n=20n (%)	Saliva n=20n (%)	DBS n=20n (%)	Saliva n=19n (%)
Diagnostic for Malignancy	11 (55)	0 (0)	11 (55)	2 (10.5)
Suspicious for Malignancy	1 (5)	1 (5)	4 (20)	2 (10.5)
ASCUS	5 (25)	10 (50)	4 (20)	7 (37)
NILM	3 (15)	9 (45)	1 (5)	8 (42)
Insufficient for evaluation	0 (0)	0 (0)	0 (0)	0 (0)

Conclusions: The cytopathological evaluation of DBS of OPC and OCC lesions may be useful as a screening and diagnostic tool and may demonstrate the presence of malignant cells. Additionally, DBS may be used for the determination of the HPV status in these patients. However, saliva specimens are not as useful as DBS, and do not appear to be a good material for screening.

377 GATA3 Expression in Malignant Effusions

Madelyn Lew, Michael Roh. University of Michigan, Ann Arbor, MI.

Background: GATA3 has been cited as a sensitive marker for breast cancers, particularly ER-positive tumors. However, studies have shown GATA3 can be positive in a small subset of triple negative cases. These findings, along with the low sensitivities of GCDFP-15 and mammoglobin, highlight the utility of GATA3 in cytology. To the authors' knowledge, previous studies of GATA3 in cytology specimens have been limited to comparisons to other breast markers, where it has been reported as more sensitive than GCDFP-15 and mammoglobin for breast cancer in effusions. However, more recent studies report GATA3 positivity in other malignancies in surgical resection specimens. The findings in these studies shed light on the limitations of GATA3 in cytology specimens, particularly in cases of unknown primaries. This projects aims to investigate the utility of GATA3 as a marker for breast carcinomas compared to mammoglobin, GCDFP15, ER, and PR and its specificity in effusions with metastatic carcinomas.

Design: A database search for pleural, pericardial, and peritoneal effusions containing metastatic carcinoma diagnosed in 2012 was performed. Available slide/cell block material was reviewed to determine adequacy (>50 tumor cells on cell block) for GATA3 IHC. Review of electronic medical records confirmed primary etiology of each case. Cases of metastatic breast cancer will be evaluated by IHC for mammoglobin, GCDFP15, ER, and PR.

Results: 136 malignant effusions were diagnosed in 2012, 94 of which had adequate material for evaluation by GATA3 IHC. Exclusion of redundant specimens yielded 81 malignant cases. 19/81 cases showed GATA3 expression

Site	Malignant Effusions (n=81)	GATA3+ (%)
Mullerian	32	2 (6)
Breast	14	13 (93)
Lung	12	2 (17)
Pancreatobiliary	7	0
Gastric	4	0
Esophageal	3	2 (67)
Colorectal	3	0
Renal	3	0
Unknown	3	0

GATA3 was positive in 13/14 breast adenocarcinomas; 11 showed diffuse expression. Diffuse GATA3 positivity was present in 4/5 ER negative breast cancers, one of which had previous negative mammoglobin and GCDFP15 stains. The last ER negative breast cancer showed focal GATA3 positivity.

Conclusions: In our analysis thus far, GATA3 has a higher rate of positivity in triple negative breast cancers than previously reported and also demonstrates that a subset of GI, pulmonary, and Mullerian primaries show a range of GATA3 positivity. Based on these findings, although GATA3 seems to be a useful and sensitive marker in identfying breast cancer in effusions, it should not be used in isolation to determine etiology.

378 Serous Cystadenoma of the Pancreas: Can an Accurate Diagnosis Possible on Cytopathology?

Mohammed Lilo, Christopher VandenBussche, Syed Ali. Johns Hopkins Hospital, Baltimore, MD.

Background: Pancreatic serous cystadenomas (SCAs) are benign tumors accounting for 1-2% of the pancreatic neoplasms. In recent decades, the availability and increasing advances in radiologic imaging has led to increased recognition of asymptomatic pancreatic cystic neoplasms and consequently an increase in endoscopic ultrasound (EUS) guided fine needle aspirations (FNA).

Design: A retrospective search of pancreatic FNAs with histological confirmation of SCA was done for an 11-year period (2004 to 2014) at a major university hospital and 58 cases were identified. Clinical data and radiologic characteristics were collected and cytomorphologic characteristics reviewed and correlated with subsequent surgical excision data.

Results: SCA ranged in size from 0.3-8.0 cm (mean: 4.9 cm). A central calcification on imaging was observed in (15%) of cases. FNA diagnoses were SCA in 5/51, suspicious for mucin-producing neoplastic cyst in 4/51, pseudocyst in 4/51 and benign ductal and/or acinic epithelium, NOS in 26/51. 12/51 cases were non-diagnostic. The salient cytomorphologic characteristics were: scant cellularity, predominantly blood, gasterointestinal contamination, hemosiderin-laden macrophages, benign ductal and acinic epithelium, mucinous columnar epithelium and cytologic atypia.

Conclusions: A cytopathologic diagnosis of SCA on FNA is extremely difficult and was only rendered in (10%) of cases in our series. Most often, the cytopathologic interpretation is descriptive or non-diagnostic (50%), related to lack of specific cellular characteristics and adequate specimen cellularity. SCA can be confused with the more commonly occurring mucin-secreting neoplastic cysts (8%) or pseudocysts (8%). The diagnosis of SCA is most often made on retrospective review after the histopathologic resection. Cytomorphologic features of SCA include: thin cystic fluid, benign-appearing cuboidal epithelium, cells with clear wispy cytoplasm, loosely cohesive cellular fragments, lack of mucinous epithelium or macrophages.

379 Fine Needle Aspiration (FNA) and Needle Core Biopsy (NCB) of Renal Lesions

Xiaoqi Lin. Northwestern University, Chicago, IL.

Background: Traditionally, most pathologic diagnoses of renal lesions were established after total or partial nephrectomy. Currently, a greater proportion are diagnosed with CT or ultrasound guided FNA/NCB in order to select suitable treatment modalities.

Design: 246 renal lesions biopsied with FNA/NCB with touch prep were retrieved.

Results: Table 1.

Lesinos	NO.	Nephrectomy (%)	Accuracy (%)	Misclassified (%)	Unclassified (%)	Atypical (%)	Unsatisfactory (%)
RCC, clear cell	75	56 (75)	67 (89)	3 (4)			5 (7)
RCC, chromophobe	11	9 (82)	9 (82)	1 (9)	1 (9)		
RCC, papillary	26	21 (81)	21 (81)	1 (4)	2 (8)		2 (8)
RCC, clear cell papillary	1	0	1 (100)				
RCC, sarcomatoid	10	5 (50)	10 (100)				
RCC, medullary	3	2 (67)	2 (67)		1 (33)		
RCC, multilocular	1	0					1 (100)
RCC, unclassified	5	3 (60)			4 (80)		1 (20)
Hybrid tumor	6	6 (100)	3 (50)	1 (17)	2 (33)		
Oncocytoma	18	4 (22)	16 (89)				2 (11)
Oncocytic tumor, unclassified	4	2 (50)			4 (100)		
Urothelial carcinoma	14	12 (86)	9 (64)			3 (21)	2 (14)
Lymphoma	10	0	10 (100)				
Metastatic malignancy	14	0	13 (93)				1 (7)
Angiomyolipoma	5	0	5 (100)				
Metanephric adenoma	1	1 (100)	1 (100)				
Spindle cell lesion	2	0	2 (100)				
Infection and inflammation	29	8 (28)	29 (100)				
Unsatisfactory	11	0					11 (100)
Total	246	129 (52)	198 (81)	6 (2)	14 (6)	3 (1)	25 (10)

Conclusions: 1. Renal biopsy has high diagnostic accuracy, 81%. Histologic examination of cellblocks or cores coupled with immunohistochemistry (IHC) significantly increases accuracy.

2. Renal biopsy significantly avoids unnecessary nephretomy, 75% for primary malignancy vs. 17% for others, and leads to chemoradiation therapy on some patients.

3. Misclassification results from overlapping of cytomorphology and/or histology and immunoprofile of clear cell RCC, chromophobe RCC, papillary RCC, oncocytoma and hybrid oncocytic tumor.

4. The causes for failing to classify these renal tumors are non-specific IHC results (64%), scant cellularity (29%) and non-specific cytomorphology (7%).

5. The cause for atypia is scant cellularity.

6. The causes for unsatisfaction are missing targeted lesions (52%) and scant cellularity (48%).

380 FNA Cytomorphology and Immunoprofile of Renal Hybrid Oncocytic Tumor

Xiaoqi Lin. Northwestern University, Chicago, IL.

Background: Hybrid oncocytic tumor is a uncommon renal tumor that shows overlapping histologic features with oncocytoma and chromophobe renal cell carcinoma (ChRCC). It was originally identified in patients with the Birt-Hogg-Dube syndrome, but can also be sporadic. The cytomorphology of fine needle aspiration (FNA) and immunoprofile of hybrid oncocytic tumor have not been well studied.

Design: Six hybrid oncocytic tumors with fine needle aspiration (FNA) possibly coupled with needle core biopsy (NCB) with touch prep were retrieved. Cytomorphology of FNA and NCB touch prep and immunoprofile of hybrid oncocytic tumor were studied and compared with those of oncocytoma or ChRCC.

Results: Large sheets of tumor cells and transversing vessels were seen more often in ChRCC than in oncocytoma ($P < 0.05$), whereas there was no statistic difference between hybrid oncocytic tumor with oncocytoma or ChRCC ($P > 0.05$). Higher nuclear grade with prominent nucleoli was seen more often in ChRCC than in hybrid oncocytic tumor or oncocytoma ($P < 0.05$), whereas there is no statistic difference between hybrid tumor with oncocytoma ($P > 0.05$). There is no statistic difference among the 3 entities in other cytologic features: architectures (small nests, single cell pattern, 3 dimensional clusters), background (myxoid-fibrous stroma), cell size and shape, cytoplasmic features (amount, granular, clear, vacuoles, membrane contours), and nuclear features (size, shape, groove, inclusion, chromatin pattern, membranous contours, binucleation, naked nuclei). No mitosis was identified. The hybrid oncocytic tumor cells are focally positive for CK7, and diffusely or focally positive for CD117. The tumor cells may be focally positive for PAX8, AMACR and CD10. Tumor cells are negative for CA IX and CK20.

Conclusions: 1. Although large sheets of tumor cells, transversing vessels and higher nuclear grade with prominent nucleoli are helpful cytologic features to distinguish ChRCC from oncocytoma, but only higher nuclear grade with prominent nucleoli is helpful to distinguish ChRCC from hybrid oncocytic tumor. In addition, there are no FNA cytologic features useful to distinguish hybrid oncocytic tumor from oncocytoma. 2. Among the tested markers, only CK7 may be useful to distinguish hybrid oncocytic tumor (focally positive) from ChRCC (more often diffusely positive) and oncocytoma (more often negative).

381 BRAF Mutations in NSCLC: Clinical Features and Outcome of a Clinical Series of Patients Diagnosed By Cytology

Maria Lozano, Jose Echeveste, Tania Labiano, Alfonso Gurpide, Jose Solorzano, Hernan Quiceno, Nerea Gomez, Pablo Preciado, Maria Amada Maset, Salvador Martin-Algarra. University of Navarra, Pamplona, Spain.

Background: Presence of genetic alterations in various kinases is known as predictive markers in non-small cell lung carcinoma (NSCLC). BRAF is one of three members of the RAF kinase family. BRAF-mutations are known as malignant drivers in a number of cancers. While BRAF mutations in NSCLC have been described, the actual prevalence and clinical features of patients with NSCLC who harbor BRAF mutations are not well defined. Therapies against the specific V600-mutated BRAF-variant are developed and show promising results.

We report a series of 205 consecutive NSCLC patients diagnosed by FNA on which BRAF mutational analysis was performed as a part of routine molecular analysis.

Design: Analysis of BRAF was introduced during last year as a part of routine molecular studies in NSCLC patients together with EGFR, KRAS, and ALK. We analyzed 205 cytological samples including 183 FNA, 8 pleural fluids, and 14 samples received for consultation. ROSE was performed in all cases to ensure a correct management of the samples. Analysis of BRAF was performed using Cobas 4800 Braf mutation test in 96 cases (46.8%), direct sequencing in 72 (35.1%), and pyrosequencing in 28 (13.7%). Nine of these cases were analyzed in duplicated by Cobas and direct sequencing.

Clinicopathological characteristics where evaluated by appropriate statistical analysis.

Results: BRAF wild type was found in 200 cases (97.6%), one was invalid due to paucity of DNA (0.5%). Four cases (2 %) harbored BRAF V600 mutation. DNA was obtained from Papanicoalou stained smears in two cases, one pleural fluid, and one cell block. Positive cases by Cobas were confirmed by direct sequencing. All harbored the V600E mutation. EGFR, KRAS and ALK were unaltered. All cases were male and smokers with stage IV adenocarcinoma. They were enrolled in a clinical trial using selective inhibitors of mutant BRAF. All four patients had partial response and three persist up to date in disease stabilization at 7,14, and 16 moths.

Conclusions: To our knowledge this is the first clinicopathological study that includes BRAF in a routine comprehensive diagnostic panel of molecular drivers in cytological samples of NSCLC. The frequency of BRAF mutation is 2%. All mutations were found in adenocarcinomas. Contrary to date reported from retrospective larger surgical series, mutations of BRAF occur in males and smokers. Clinical trails show promising results in BRAF mutated patients. This study supports the value of cytological samples for molecular panels. Adequate management of samples is mandatory.

382 Accurate Diagnosis of Angioimmunoblastic T-Cell Lymphoma By Fine Needle Aspiration Is Feasible When Combined With Flow Cytometry and Cell Block-Based Immunocytochemistry: A Correlative Study With Concurrent Core Needle Biopsies

Shannon Mann, Anna Richmond, Jeffrey Jorgensen, Roberto Miranda, Ruth Katz. University of Texas MD Anderson Cancer Center, Houston, TX; Methodist Hospital, Houston, TX.

Background: Mature T-cell lymphomas are difficult to diagnose on small biopsies. Angioimmunoblastic T-cell lymphoma (AITL), the most common peripheral T-cell lymphoma, is particularly difficult to diagnose due to its complex morphologic, immunophenotypic and molecular findings. Follicular dendritic cell (FDC) hyperplasia and association with Epstein Barr Virus are common. The lymphoma cells show a follicular T-helper phenotype, positive for CD10, CXCL13 and PD-1. Moreover, immunophenotypic aberrancies of T- and B-cells can be detected by multiparameter flow cytometry (MFC).

Design: We performed a retrospective study to evaluate the accuracy of fine needle aspiration (FNA) diagnosis on patients with AITL as compared with a concurrently acquired core biopsy. Between 2005 and 2014, sixteen cases of AITL were diagnosed by FNA at our institution. We reviewed FNA slides, cell blocks (CB) prepared from FNAs and concurrent core biopsies (Cbx) with accompanying MFC and immunohistochemistry (IHC).

Results: The pathologic findings on CBs relative to Cbx were generally excellent with almost complete diagnostic concordance between FNAs and Cbx. Results of subsequent IHC studies, performed on both CB and Cbx were identical, with positive staining for CD10, CXCL13, PD-1 and CD21. EBER was demonstrated in most cases. Aberrant T- and B-cell phenotypes were identified by MFC in 13/16 and 2/16 cases respectively. There were 2 cases on follow up of EBV-positive large B-cell lymphoma, arising in a background of AITL. On follow up, 8/16 patients were dead and 3/16 patients were alive with disease.

Conclusions: AITL is an aggressive disease with a poor prognosis. We show that FNA is a good diagnostic modality for diagnosing AITL provided sufficient tissue is obtained for CB, and appropriate ancillary studies are performed. This approach is facilitated by rapid on-site assessment to ensure adequacy of aspirated tissue and appropriate triage. All modalities, morphology IHC and MFC are essential in achieving the correct diagnosis of AITL and excellent concordance between cytology, CB and Cbx was noted.

383 Accuracy and Interobserver Reliability of the Cytologic Diagnosis of Low Grade Urothelial Carcinoma (LGUC) in Instrumented Urinary Tract Cytology Specimens

Zulfia McCroskey, Stephanie Kleitherms, Tatjana Antic, Michael Cohen, Guliz Barkan, Stefan Pambuccian, Eva Wojcik. Loyola University Medical Center, Maywood, IL; University of Chicago, Chicago, IL; University of Utah, Salt Lake City, UT.

Background: The inclusion of LGUC as a distinct category in the new classification scheme ("The Paris System") is controversial. The aim of this study was to investigate the accuracy and interobserver agreement of LGUC cytologic diagnoses.

Design: Forty bladder barbotage and upper urinary tract specimens (19 with corresponding biopsies diagnosed as LGUC and 21 with negative biopsies during an average 26 months followup interval) were blindly reviewed by 6 experienced cytopathologists from 3 institutions, who diagnosed them as NEGATIVE FOR MALIGNANCY (NM), ATYPICAL UROTHELIAL CELLS (AUC), SUSPICIOUS FOR LGUC (SLGUC) and LGUC. Interobserver agreement was measured with the weighted Kappa (*K*) statistic. Using the corresponding biopsy as a gold standard, two-by-two tables were constructed. Diagnoses of NM, AUC were considered negative. Diagnoses of ³SLGUC were considered positive. Each observer's sensitivity, specificity, PPV and NPV were calculated.

Results: The mean sensitivity for LGUC was 41% (range 21-53%).

Reviewers	Sensitivity(%)	Specificity(%)	PPV(%)	NPV(%)	Correct Diagnosis(%)
1	32	76	55	55	55
2	21	95	80	57	60
3	53	71	63	63	63
4	47	67	56	58	58
5	47	95	90	67	73
6	47	81	69	63	65

Across all raters, for all result types, agreement among the reviewers was fair (*K* = 0.23); agreement was better for NM (*K* = 0.36), slight for LGUC (*K* = 0.14) and lowest for AUC (*K* = 0.07).

Cytologic Diagnoses	Kappa	95% Confidence Intervals	p
Overall agreement	0.23	0.18 – 0.28	
NM	0.14	0.28 – 0.44	
SLGUC	0.18	0.10-0.26	
LGUC	0.14	0.6-0.22	
AUC	0.07	-0.01 – 0.15	

Pairwise inter-rater reliability (weighted Kappa) for the test result.

Reviewers	1	2	3	4	5	6
1	--	0.43(0.12)	0.40(0.10)	0.46(0.09)	0.29(0.13)	0.42(0.11)
2	--	--	0.28(0.11)	0.38(0.11)	0.27(0.15)	0.36(0.15)
3	--	--	--	0.46	0.30	0.36(0.15)
4	--	--	--	--	0.22(0.11)	0.26(0.11)
5	--	--	--	--	--	0.17(0.14)

Conclusions: Only slight agreement was observed in cases of LGUC (*K*= 0.14) and the agreement was low even between the reviewers from the same institution.

384 Eliminating of the "Suspicious for High Grade Urothelial Carcinoma" (SHGUC) Category Results in Unacceptable Loss of Sensitivity for High Grade Urothelial Carcinoma (HGUC) Diagnosis in Urinary Tract Cytology (UTCy)

Zulfia McCroskey, Grazina Chatt, Reza Eshraghi, Burak Bahar, Eva Wojcik, Stefan Pambuccian, Guliz Barkan. Loyola University Medical Center, Maywood, IL.

Background: Efforts to standardize the nomenclature in reporting urine cytology by an international group of experienced and dedicated cytopathologists are currently underway ("The Paris System"). One of the proposed diagnostic categories within this system is SHGUC. The aim of our study was to evaluate inter-observer concordance of this cytologic diagnosis and to determine its impact on the sensitivity of urinary tract cytology for HGUC.

Design: UTCy specimens, originally diagnosed as HGUC (n=15), "Atypical urothelial cells" (n=15), "Negative for malignancy" (NM), and "polyomavirus changes" (PyV) (n=20) were identified from the files of our institution. All cases of HGUC had corresponding confirmatory biopsies, whereas all AUC cases had negative follow-up. 10 observers (3 cytotechnologists, 2 residents and 5 cytopathologists) reviewed the 50 specimens and classified them as NM, PyV, SHGUC and HGUC. The diagnoses made by the observers were compared to the biopsy result (gold standard).

Results: Complete agreement for the reference diagnosis of HGUC varied between 10-100% (mean 78% +/-27.8%) and at least partial agreement (SHGUC and above) varied between 70-100% (mean 96% +/-8.2%). SHGUS diagnoses showed an unexpectedly high concordance between reviewers (68% +/-25.6%).

Reviewers	% of agreement on HGUC diagnosis mean (range)	% of agreement on at least SHGUC diagnosis mean (range)	Sensitivity at SHGUC mean (range)	Sensitivity at HGUC mean (range)	Specificity at SHGUC mean (range)	Specificity at HGUC mean (range)
Cytotechnologists (n=3)	78 (0-100)	98 (67-100)	91 (80-100)	78 (67-87)	98 (95-100)	98 (95-100)
Residents (n=2)	80 (0-100)	78 (0-100)	97 (93-100)	77 (73-87)	78 (75-80)	78 (75-80)
Cytopathologists (n=5)	77 (20-100)	86 (20-100)	99 (93-100)	80 (67-80)	86 (75-95)	86 (75-95)
All observers mean	78	87	96	78	87	87

HGUC: high grade urothelial carcinoma; SHGUC: suspicious for high grade urothelial carcinoma; n: number of reviewers.

Conclusions: Including a suspicious category resulted in 96% (80-100%) and 87% (75-100%) sensitivity and specificity rates respectively. If the SHGUC category would not have been used, these numbers would have dropped to 78% (66.7-86.7%) sensitivity and 87% (75-100%) specificity. As such, we strongly believe that SHGUC category should be included in the "Paris system".

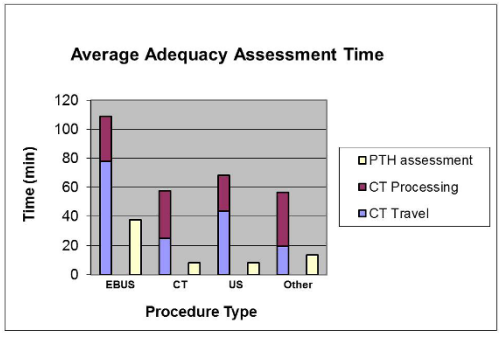
385 Utilization Review and Reimbursement of Cytology Services in Endobronchial Ultrasound Guided Procedures: Challenge and Opportunity

Ross Miller, Angela Wright, Laura Satrum, Dina Mody, Mary Schwartz, Michael Thrall. Houston Methodist, Houston, TX.

Background: The roles of pathologists (PTH) and cytotechnologists (CT) continue to evolve in order to optimize patient care, particularly with regard to rapid on-site evaluations (ROSE). Having ROSE performed helps ensure sufficient material is obtained for diagnosis and permits specimen triage for ancillary studies. At our institution, both on-site and telecytology evaluations are increasingly utilized, particularly in endobronchial ultrasound guided procedures (EBUS). Consequently, time demands placed on the PTH and CT staff have exponentially increased creating workload management challenges.

Design: A representative number of ROSE were documented for a three month period at our instituion. Case type and time spent for travel, adequacy assessment, processing, screening, and sign out was recorded in order to assess time demands placed on staff by different procedures.

Results: Average travel/processing time by CTs was variable among procedures (72.9min) as was adequacy assessment time by PTH (16.9min). EBUS posed the greatest time challenges with the longest travel/processing times (109min) and longest adequacy assessment time (37.8min).



CT travel/processing time for EBUS takes almost 40% longer and adequacy assessment time takes the PTH 3-4x longer when compared to other procedures due to the targeting of multiple sites during EBUS with associated procedural delays. Using telecytology, PTH time was reduced from 44.8 min to 24.6 min for EBUS. The provision of ROSE for EBUS is more challenging from a workload management perspective than other procedures.

EBUS Procedure Cost and Reimbursement				
	Technical Component	Professional Component	Global Component	Net Gain/Loss
Reimbursement	$46.73	$113.97	$160.70	
Cost (using telecytology)	$109.00	$77.99	$186.99	-$26.29
Cost (non-telecytology)	$109.00	$142.03	$251.03	-$90.33
Cost (overall average)	$109.00	$119.84	$228.84	-$68.14

Conclusions: ROSE reimbursement is low, and no greater for EBUS than other procedures. Use of telecytology can save time for PTH and make the service more cost-effective if the number of procedures is sufficient to justify investment in the technology.

386 SOX10/Cytokeratin Dual-Color Immunohistochemistry Is an Effective First-Line Test for the Work-Up of Epithelioid Malignant Neoplasms in Fine Needle Aspiration and Small Biopsy Specimens

Jeffrey Mito, James Conner, Jason Hornick, Edmund Cibas, Xiaohua Qian. Brigham & Women's Hospital, Boston, MA; Harvard Medical School, Boston, MA.

Background: Characterization of poorly differentiated tumors in small biopsy and fine needle aspiration specimens usually requires immunohistochemistry (IHC) with a panel of markers. Because of an increasing need to preserve limited diagnostic material for molecular studies to guide targeted therapies, and a mounting demand for cost containment, we investigated the usefulness of dual-color IHC with AE1/AE3, a broad spectrum cytokeratin, and SOX10, a recently characterized neuroectodermal transcription factor consistently expressed in melanoma, in the work-up of epithelioid malignant neoplasms.

Design: In total, 89 cases of small biopsies (n=46) and fine needle aspiration cell blocks (n=43), for which S100 and AE1/AE3 IHC was performed in the initial work-up, were selected, including 30 melanomas, 27 epithelioid/pleomorphic sarcomas (gastrointestinal stromal tumors, epithelioid angiosarcoma, proximal-type epithelioid sarcoma, undifferentiated pleomorphic sarcomas and others), and 32 poorly differentiated carcinomas. IHC was performed on all specimens using a peroxidase-based brown chromogen for SOX10 and an alkaline phosphatase-based red chromogen for AE1/AE3. The presence or absence of staining in lesional cells was scored.

Results: Most tumors showed one of three distinctive patterns: A) malignant melanomas with nuclear SOX10 alone (28 of 30; 94% sensitivity and 92% specificity); B) epithelioid/pleomorphic sarcomas negative for both SOX10 and AE1/AE3 (21 of 27; 78% sensitivity and 92% specificity), except for clear cell sarcomas (n=2) and epithelioid malignant peripheral nerve sheath tumor (n=1), which showed only nuclear SOX10 staining; and C) poorly differentiated carcinomas with cytoplasmic AE1/AE3 alone (29 of 32, 91% sensitivity and 98% specificity). The number of immunostains/slides used to reach a diagnosis of malignant melanoma initially was 5.5 (mean; range 2-13), compared to only one slide with 2 stains using SOX10/cytokeratin dual-color labeling.

Conclusions: SOX10/cytokeratin dual-color IHC is a sensitive and specific test to distinguish between melanoma, sarcoma and carcinoma. This approach can effectively identify melanoma, prioritize a more focused panel, and limit the number of markers needed to work-up an epithelioid malignant neoplasm – potentially reducing the cost of IHC and preserving valuable tissue for molecular studies.

387 Examining the Utility of Pelvic Washing Cytology in Endometrial Cancer Staging: Is This Procedure Valid?

Anna Nam, Rana Hoda, Edyta Pirog, Susan Alperstein, Tamar Giorgadze. New York Presbyterian Hospital – Weill Cornell Medical College, New York, NY.

Background: The surgical staging of endometrial carcinoma was modified by the International Federation of Gynecology and Obstetrics (FIGO) in 2009. Among other changes, the 2009 FIGO system excluded positive pelvic washings (PWs) from the staging criteria, whereas by the prior 1988 guidelines, positive PWs resulted in a clinical stage of at least IIIa. The current FIGO guidelines recommend a separate reporting of the cytology result. Therefore, intraoperative PWs continue to be performed, resulting in a significant expenditure of healthcare cost, time and energy. To determine whether the PWs affect clinical outcome to justify its continued use, a 5-year retrospective study was conducted examining the frequency of positive PWs in stage I or II carcinoma by the 2009 guidelines and their outcome.

Design: The cohort was selected by reviewing pathology reports of all patients who underwent surgeries for endometrial cancers with intraoperative PWs from 1/2008-12/2012. Patients who had a 2009 FIGO stage I or II with positive PW cytology were included in the case group. The control group comprised of all the patients with stage I or II disease and negative PW. The outcome measure of interest was disease progression (recurrence, metastasis, or death).

Results: Of the 50 patients in the cohort, 15 patients had positive PW cytology (30%), 1 atypical cytology (2%), and the rest negative (68%). Of the patients with positive cytology, 6 had a stage I/II disease (or, if staged in 2008-2009, had a stage IIIa by the 1988 criteria but would have been staged I/II by the 2009 system) (8.3% of total). Of these 6 patients with stage I/II disease and positive PW, 1 patient had disease progression (16.7% of cases), compared to 3 out of 28 patients (10.7%) in the control group ($c^2(1, N = 34) = 0.17$, $p = 0.68$). The clinical information and histological findings for each group are summarized in the table.

	Case group (stage I/II+PW)	Control group (stage I/II –PW)
Number of patients	6	28
Mean age (years)	71.5	64
Follow-up period (months)	34	33.5
Stage I (%)	83	93
Adjunctive therapy (%)	66.7	53.6
Histological types (%):		
Endometrioid	50	60.7
Serous	33.3	14.3
MMMT	16.7	17.9
Poorly-differentiated	0	7.1
Lymphovascular invasion (%)	50	25
Disease progression (%)	16.7	10.7

Conclusions: This data suggests that PWs may not provide significant additional prognostic information and its routine use may not be indicated.

388 Current Role of Fine-Needle Aspiration of Axillary Lymph Nodes in the Management of Early-Stage Breast Cancer

Nicole Nelles, Savitri Krishnamurthy. University of Texas MD Anderson Cancer Center, Houston, TX.

Background: Because clinical practice is rapidly evolving in patients with early-stage breast cancer, the role of fine-needle aspiration (FNA) of axillary lymph nodes (ALNs) in initial staging in this patient population is unclear. We evaluated the current utility of ultrasound (US)-guided FNA of ALNs in the initial staging of early-stage breast cancer.

Design: We reviewed the records of all patients with stage T1 or T2 breast cancer who underwent US-guided FNA of indeterminate and suspicious ALNs at a single institution between July 2013 and June 2014. US-guided FNA was performed using a 23-gauge needle, and specimens were fixed in alcohol or air-dried for Papanicolaou and Diff-Quik staining; results categorized as negative or positive for metastatic carcinoma. We examined the relationship between FNA results and the final axillary status determined following definite surgery. The sensitivity, specificity, and false negative rate of US-guided FNA of ALNs, as well as the implications of the results in the selection of patients for sentinel lymph node (SLN) biopsy or axillary dissection (AD), were determined.

Results: A total of 180 patients with early-stage breast cancer underwent US-guided FNA of ALNs. FNA results were positive for metastatic carcinoma in 49 cases (27%) and negative in 134 cases (73%). Among patients with negative FNA results, 98 (73%) underwent SLN biopsy, 6 (4%) underwent AD, and 23 (17%) underwent both. Metastatic carcinoma in the SLNs was noted in 35 patients in this group (25%). The mean size of the metastatic carcinoma was 0.3 cm (0.025-1.25 cm), involving 1 SLN in 26 patients (74%) and 2 SLNs in 7 patients (20%). Among patients with positive FNA results, 33 (68%) underwent AD, 3 (6%) underwent SLN biopsy, and 11 (22%) underwent both. The mean size of the metastatic carcinoma was 1.1 cm (0.5-3 cm), involving 1 SLN in 7 patients (88%) and 2 SLNs in 1 patients (12%)**.** The sensitivity of FNA for initial staging was 69%, the specificity was 100%, and the false negative rate was 31%.

Conclusions: 1. The results of US-guided FNA of ALNs had significant clinical implications for the management of axilla in patients with early-stage breast cancer.
2. Most patients with negative FNA results underwent SLN biopsy (73%), and most patients with positive results (68%) underwent AD.
3. The limited burden of metastatic tumors in patients with positive FNA results (usually only 1 positive SLN, 88%) suggests that only the positive lymph node identified by FNA could be targeted, rather than subjecting the patient to complete AD.

389 Predictive Value of HPV Testing for High-Grade Cervical and Vaginal Intraepithelial Lesions in Patients With Mildly Abnormal Pap Testing Results and Therapy Effects

Nicole Nelles, Abha Khanna, Shobha Patel, Yun Gong, Ming Guo. University of Texas MD Anderson Cancer Center, Houston, TX.

Background: Therapy for cancer patients, including chemotherapy and radiation, can cause cytologic atypia in Pap specimens that may pose diagnostic challenges. To evaluate whether HPV testing can help predict high-grade cervical or vaginal intraepithelial lesions (CIN/VAIN2+) in Pap specimens for patients during post-therapy follow-up, we conducted a retrospective review for these patients with mildly abnormal Pap testing results and therapy effects.

Design: From 2007 to 2013, we retrospectively reviewed follow up data from 177 patients who had chemo/radiation therapy and Pap test with mildly abnormal Pap results (155 of ASC-US, 13 of ASC-H and 9 of LSIL) and therapy effects. The Pap specimen consisted of 87 cervical and 90 vaginal specimens. Patient's age ranged from 19-87 with an average of 53 years. Of 177 patients, 90 had a history of cervical or vaginal carcinoma. HPV testing (Hybrid Capture 2 or Cervista HPV) was performed. The follow-up data (average 26 months) including 58 surgical cases (26 cervical/endocervical, 31 vaginal and one uterus), 119 Pap tests and 91 HPV tests were compared with HPV status.

Results: Of 177 patients, HPV was positive in 51 (28.8%). Twenty-one patients (12%) had a follow up result of either HSIL (4) or CIN2+/VAIN2+ (17). Of 126 patients with a negative HPV testing result, follow-up showed 2 HSIL and 7 CIN2+/VAIN2+. In 39 patients with a positive HPV testing results, 2 had HSIL and 10 had CIN2+/VAIN2+ follow up results. The difference of risk between the 2 groups was significant (P=0.004).

Follow-up for Patients withMildly Abnormal Pap Testing Results and Therapy Effects			
	≤CIN/LSIL	≥CIN2/HSIL	Total
HPV+	39	12	51
HPV-	117	9	126
Total	156	21	177
p=0.004 (Fisher's Test)			

In 91 patients who had HPV follow-up tests, patients with positive follow-up HPV testing had a significantly higher risk of HSIL/CIN2+/VAIN2+ than those with negative follow-up HPV testing results, i.e. 33% (7/21) vs 7% (5/70) (P=0.005).

Conclusions: A positive HPV testing result predicted a significantly higher risk of HSIL/CIN2+/VAIN2+ for patients with mildly abnormal Pap results and therapy effects during post-therapy follow up. However, Pap and HPV co-testing may still be necessary for patients with mildly abnormal Pap results and therapy effects during post-therapy follow-up due to relatively high false negative HPV testing results.

390 EBUS-TBNA Yields Multiple Gene Analyses and a Panel of Immunohistochemical Stains on Routinely Prepared Cell Blocks in Non-Small Cell Lung Cancer Cases

Olga Nikolskaia, Zahra Maleki. Johns Hopkins University, Baltimore, MD.

Background: Endobronchial ultrasound-guided transbronchial needle aspiration (EBUS-TBNA) is a non-invasive tool for diagnosis and staging of non-small cell lung carcinoma. Samples are often used for molecular studies to evaluate mutations in lung adenocarcinoma for therapeutic and prognostic purposes. However, the value of this approach to obtain a final diagnosis without need for subsequent sampling has not been addressed. Here, cases for which EUS-TBNA was conducted from December 2013 - May 2014 were retrieved to determine the success of this method toward a complete diagnosis that does not require subsequent re-sampling or invasive approaches to resolve uncertainties in the EBUS-TBNA.

Design: All EBUS-TBNA cases with malignant final diagnoses that were performed at the Johns Hopkins Hospital between December 2013 and May 2014 were identified. Patient demographics, final diagnoses, number of aspirated sites, sampled locations, on-site evaluation of sample adequacy, and immunohistochemistry (IHC) and molecular study results were recorded for each case.

Results: 75 EBUS-TBNA specimens were identified. All cases had on-site evaluation performed and cell block made from needle rinses. No core biopsies were taken. IHC was performed on 48 cases (66%). Each had 1-14 immunostains (mean 4.3). TTF-1 (47) was the most frequently ordered stain, followed by Napsin A (37) and p40 (29). Molecular testing for lung carcinoma was performed for 59 of 73 (81%) cases, while 9 of 73 cases (12%) had insufficient sample for molecular studies. Twenty seven cases showed mutations on molecular studies, including KRAS (13), EGFR (7), ALK (3), and one each for BRAF, ERBB2, NRAS, and KIT. Final diagnoses were rendered based on EBUS-TBNA or existing biopsy material alone and additional sampling procedures were not required.

Conclusions: These findings demonstrate that EBUS-TBNA is a valuable tool in obtaining adequate material for a definitive diagnosis of lung non-small cell carcinomas. A cell block prepared from needle rinses can provide sufficient material for 1) an H&E-stained slide, 2) molecular analysis of mutations in multiple genes, 3) evaluation of ALK translocation by FISH, and 4) a panel of immunohistochemical stains in the majority of cases. EBUS-TBNA could be easily repeated to collect additional material for molecular studies in cases with inadequate material, but was deemed unnecessary in this series.

391 The Value of Multiple Negative Peritoneal Fluid Cytology Specimens in Patients With No Prior History of Malignancy

Jacqueline Nunez, Shweta Patel, Maria Muniz, Jan Silverman. Allegheny General Hospital, Pittsburgh, PA.

Background: Peritoneal fluid cytology is utilized for the evaluation of effusions of the abdominal cavity with its primary value to diagnose malignancy. It is not uncommon to evaluate multiple peritoneal fluid cytology specimens in the management of patients with ascites. To the best of our knowledge, the diagnostic yield of repeated peritoneal fluid analysis in patients with negative cytologies and no prior history of malignancy has not been studied. The purpose of this study is to evaluate the clinical utility of multiple peritoneal fluid cytology on patients with no prior history of malignancy in a one year time period.

Design: A total of 1,143 cases were reviewed with peritoneal fluid cytology evaluations in a one year period at our institution. We defined multiple cases as having three or more negative cytology specimens. We only included cases that had an initial negative result and no prior history of malignancy during this one year period.

Results: From the initial pool of 1,143 cases, 67 (6%) of the cases had an initial negative cytology report and multiple repeated negative cytology interpretations. The range in age was 29-82 years (median of 57 years), the sex distribution was 17 females and 50 males. The range of repeated submitted peritoneal fluid cytology specimens was from 3-31 (mean of 5). The etiologies of 66 multiple negative cases was liver cirrhosis, and 1 cases of polycystic kidney disease with cystic liver disease.

Conclusions: In this one year review of perotineal fluid cytology in patients with no prior history of malignancy, there was no patient in which repeated cytology specimens diagnosed malignancy. Therefore, although paracentesis has a therapeutic value, submitting specimens for cytologic examination has limited utility for the diagnosis of malignancy.

392 Follow-Up of Negative Pap Tests in Patients With Abnormal Clinical Signs or Symptoms: A Large Single Institution Study

Rebecca Ocque, R Marshall Austin. University of Pittsburgh Medical Center, Pittsburgh, PA.

Background: Abnormal signs or symptoms may be documented in women having Pap testing and have often been seen in our Pap test litigation consultations. Clinical assessment is important even with a negative Pap test interpretation, as findings may reflect significant undetected disease. Since 2009, we have specifically reported such patients with a novel designation: "Negative for Intraepithelial Lesion or Malignancy, Abnormal Clinical Signs or Symptoms." These Paps are routinely submitted for rescreening by a second quality control cytotechnologist and for pathologist review. The objective of this study was to document available follow-up findings in our laboratory information system (LIS).

Design: A search for patients with Pap test results of "Negative for intraepithelial lesion or malignancy, abnormal clinical signs or symptoms" was performed from 1/1/2009 to 10/1/2013. Clinical information and follow-up screening test results and histopathologic findings were documented.

Results: 1104 cases were identified during the study period. The average age was 50 (range 16-92). Most common signs and symptoms were abnormal bleeding in 891 (81%), polyps in 83 (8%), pelvic mass in 54 (5%), visible cervical lesions in 48 (4%), vaginal lesions in 17 (2%), and endometrial masses in 6 (0.5%). Of the 1104 cases, 667 (60%) had follow-up results in our LIS, including 517 with histopathologic diagnoses. Of the 517, 233 (45%) had nonspecific benign diagnoses, 216 (42%) had benign tumors or tumor-like conditions, 28 (4%) had insufficient tissue for diagnosis, 17 of 571 (3%) had precancerous diagnoses, and 23 (4%) had malignant diagnoses. Among 23 malignant histopathologic diagnoses, average time to diagnosis was 128 days. The primary site of malignancy was endometrial in 14 (61%), ovarian in 5 (22%), and miscellaneous in 4 (17%). No cervical malignancies were identified. Recently we also saw two cervical cancer patients with negative HPV results, abnormal signs and symptoms, and abnormal cytology.

Conclusions: We report here for the first time follow-up findings for a large cohort of patients with negative Pap test results and abnormal clinical signs or symptoms. 23 of 571 (4%) patients with follow-up histopathologic findings had malignant diagnoses, most frequently endometrial malignancy. Absence of cervical malignancies in this cohort supports the effectiveness of quality control rescreening. Abnormal clinical signs and symptoms should also be reported in patients with negative HPV test results.

393 The Reliability of a Cost-Effective Eyepiece Microscope Camera for HIPAA-Secure Dynamic Telecytopathology

Damon Olson, Lindsay Shearer, Judy Harper, Sherif Said, Loes Nardi-Korver, Amy Storfa. Denver Health Medical Center, Denver, CO.

Background: Rapid on-site evaluation (ROSE), in which cytopathologists perform adequacy assessments, render preliminary diagnoses, and recommend adjunct testing is performed during fine needle aspiration (FNA) procedures. Physically leaving the office to perform FNA assessments can be burdensome. The use of expensive microscope cameras that can transmit live video feed to pathologists with access to a computer and the internet (dynamic telecytopathology) has been shown to reduce the time it takes to perform ROSE and to be highly concurrent with on-site evaluation. The use and reliability of a cheaper eyepiece camera has yet to be described in the literature.

Design: Three pathologists that regularly perform ROSE at our institution were twice taken through different sets of six representative sample cases retrieved from our FNA archives. First, each pathologist reviewed the case in real time on his or her computer screen while verbally guiding a certified cytotechnologist controlling the slide movement. Video feed was captured by a $600 microscope eyepiece camera and transmitted via secure intranet video conferencing software. Each pathologist was asked to assess the specimen adequacy, if additional passes were needed, if the case was benign or malignant, what their preliminary diagnosis would be, and if samples should be sent for flow cytometry and microbiology studies. The pathologists were then asked to re-review the same cases under direct microscopic examination and asked the same questions.

Results: 18 sample cases were reviewed. Adequacy assessments were unchanged in 17 and all additional pass requests remained unchanged. A determination of benign versus malignant was deferred in two cases (11%). Preliminary diagnoses were further refined during the second round in four cases (22%). A request for flow cytometry was reversed in one case (6%) and requests for microbiology studies were unchanged in all cases.

Conclusions: As assessment complexity increased, agreement between rounds diminished slightly. Adequacy assessments were unchanged in 94%, 89% in the assessment of benign versus malignant, and to 78% when establishing a preliminary diagnosis. The 94% of these changes were refinements of the initial impressions established in the first round. Relatively inexpensive microscope eyepiece cameras may provide a reliable alternative to physical ROSE and transmission through a hospital's internal communication network ensures protection of patients' private health information.

394 Atypical Urothelial Tissue Fragments in Non-Instrumented Voided Urine Specimens Are Associated With Low Rates of Urothelial Neoplasia

Irem Onur, Dorothy Rosenthal, Chris VandenBussche. Johns Hopkins Medical Institutions, Baltimore, MD.

Background: The interpretation of urothelial tissue fragments (UTF) in voided urine (VU) specimens is controversial. If UTF contain cytomorphologically atypical cells, the diagnosis may become more challenging.

Design: We searched the electronic pathology database system for VU cases containing UTF for a 5-year period (2009-2013). We previously analyzed benign-appearing UTF (BUTF) in 274 non-instrumented VU specimens. In this study, non-instrumented VU containing atypical UTF (AUTF) which resulted in an atypical-category diagnosis were evaluated and compared with the previous analysis.

Results: The search revealed 170 cases of non-instrumented VU containing AUTF. Of these, 24 had surgical pathology specimens during the follow-up period which showed the following overall rates of neoplasia: High-grade urothelial carcinoma (HGUC) (8.8%; n=15), low-grade urothelial neoplasia (LGUN) (1.2%; n=2), and prostate carcinoma invading bladder (0.6%; n=1). Forty-eight cases (28.2%) were found to have recent stones on follow-up. Of 73 cases without histopathologic, macroscopic, radiologic or cytopathologic follow-up, 62 cases (84.9%) had a mean clinical follow-up period of 22.5 months (range: 1 to 59 months).

TABLE 1. Follow-up of cases of non-instrumented VU containing BUTF and AUTF

Follow-up	Number of cases of non-instrumented VU containing BUTF (%)	Number of cases of non-instrumented VU containing AUTF (%)
Histopathologic	29 (10.6)	24 (14.1)
Benign	17 (6.2)	6 (3.5)
Urothelial neoplasia	12 (4.4)	17 (10.0%)
LGUN	10 (3.6)	2 (1.2)
HGUN	2 (0.7)	15 (8.8)
Macroscopic/Radiologic/ Cytopathologic/Clinical	220 (80.3)	135 (79.4)
Urinary tract stone	45 (16.4)	48 (28.2)
Benign cytology	45 (16.4)	25 (14.7)
No follow-up	25 (9.1)	11 (6.5)

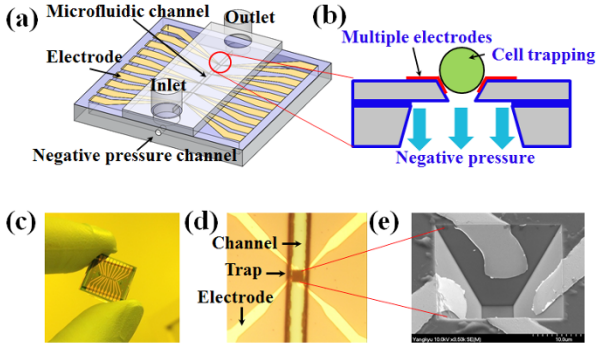
Conclusions: The presence of AUTF in non-instrumented VU is associated with low rates of urothelial neoplasia but indicates a higher risk of urothelial neoplasia than the presence of benign-appearing UTF (10.0% vs 4.4%; $p<0.05$). In particular, the rate of HGUC is significantly higher in non-instrumented VU containing atypical UTF (8.8%) than those containing BUTF (0.7%; $p<0.0001$). It is important to keep in mind that urinary tract stones are associated with AUTF in a substantial proportion of non-instrumented VU specimens.

395 Discrimination Between Normal and Cancerous Human Urothelial Cell By Using a Novel Electrical Impedance Spectroscopy With Three-Dimensional Multiple Electrodes on a Trap

Yangkyu Park, Joho Yun, Hyeon Woo Kim, Giseok Kang, Seungwan Seo, Jonghyun Lee. Gwangju Institute of Science and Technology, Gwangju, Republic of Korea.

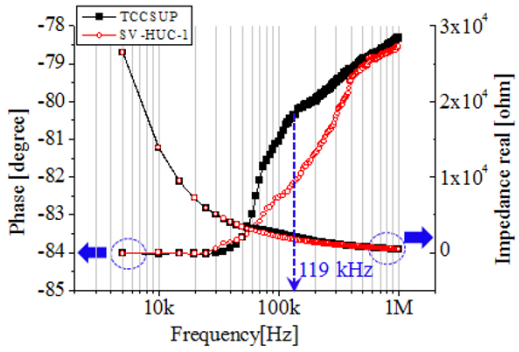
Background: Micro electrical impedance spectroscopy (μEIS) sensitively discriminates cells in different physiologic state, for instance, normal and cancerous cells. The present study estimated the electrical impedance difference of normal (SV–HUC–1) and cancerous (TCCSUP) human urothelial cell lines using a novel μEIS.

Design: The proposed μEIS consists of three parts: a fluidic channel, sensing electrodes and a negative pressure channel for cell trapping (Fig.1–a). While the cells are captured in a trap, the sensing electrodes can detect the electrical properties (Fig.1–b). The electrical impedance could be measured with high sensitivity, which is attributed to the tight contact between the cell and the electrodes due to the negative pressure. Micromachining process was used to fabricate the device (Fig.1–c,d,e). SV–HUC–1 and TCCSUP were cultured and injected into the fluidic channel. When the cells were captured, electrical impedance was measured 30 times by impedance analyzer (frequency range: 5kHz–1MHz).



[Fig.1]

Results: Electrical impedance consists of magnitude and phase, or real and imaginary part. In this study, the clearest difference of the average impedance was observed in phase and real part between the two cell lines at 119kHz (Fig.2). Statistically significant differences were shown ($p<0.05$) in the mean value of phase (SV–HUC–1:-82.39±0.01° vs TCCSUP:-80.57±0.04°) and real part (SV–HUC–1:1822.89±3.42Ω vs TCCSUP:2246.82±9.43Ω). The maximum differences of mean phase and real part were 1.82° and 423.93Ω, respectively at 119kHz (Fig.2).



[Fig.2]

Conclusions: We suggest this fabricated μEIS can be applied for supplementary tool in detecting urothelial cancer with urinary specimen.
[This work was financially supported by IMSE].

396 Significance of HR-HPV Detection in Women With AGC on Pap Test: Analysis of 1857 Cases From One institute

Stell Patadji, Dinesh Pradhan, Chengquan Zhao. University of Pittsburgh Medical Center, Pittsburgh, PA.

Background: The role of hrHPV testing in women with various types of AGC on Pap test is undetermined.

Design: To search for AGC Pap test in the database from 2008-1 to 2013-12. Cases of AGC with concurrent hrHPV testing and histological follow-up within one year were included.

Results: 2287 women with AGC Pap had concurrent HC2 HPV testing results, constituting 1287 AGC-NOS, 619 AEC, 96 AMC, 12 AGC-F, 217 AGC/ASC-H, 32 AGC/LSIL, 24 AGC/HSIL. The overall HPV+ rate was 27.7%. Within one year after the AGC Pap test, 1857 cases (81.2%) had histological follow-up, of which 529 had a hrHPV+ test with a mean age of 38.2 y, while 1328 had a hrHPV- test with a mean age of 44.7 y. Among the HPV+ cases, 16.7% had CIN2/3, 5.3% had cervical AIS/ADC, and 1.5% had an endometrial carcinoma. Among the HPV- cases, 0.6% had CIN2/3, 0.2% had AIS/ADC, and 3.4% had an endometrial carcinoma. All 82 AMC cases were HPV- and 13.4% of which developed endometrial carcinoma. When the patients were divided into three age groups, the rate of cervical CIN2/3/AIS/ADC was significantly higher in hrHPV+ women than that in hrHPV- women in all age groups. Endometrial carcinoma was most commonly present in women aged 50 years and older, especially in hrHPV- women.

AGC Types	HPV result (No)	CIN1 (%)	CIN2/3 (%)	AIS/ ADC (%)	EM Hyperplasia (%)	EM Ca (%)	Metastatic Ca (%)
AGC*	Pos 222 Neg 828 P+N 1050	65 (29.3) 83 (10.0) 148 (14.1)	26 (11.7) 4 (0.5) 30 (2.9)	16 (7.2) 016 (1.5)	09 (1.1) 9 (0.9)	7 (3.2) 37 (4.5) 44 (4.2)	1 (0.5) 3 (0.4) 4 (0.4)
AEC	Pos 145 Neg 353 P+N 498	37 (25.5) 53 (15.0) 90 (18.1)	14 (9.7) 1 (0.3) 15 (3.0)	8 (5.5) 0 8 (1.6)	1 (0.7) 3 (0.9) 4 (0.8)	000	0 0 0
AMC	Pos 0 Neg 82 P+N 82	04 (15.0) 4 (4.9)	0 0 0	0 1 (1.2) 1 (1.2)	0 8 (9.8) 8 (9.8)	011 (13.4) 11 (13.4)	00 0
AGC/ ASC-H	Pos 121 Neg 58 P+N 179	40 (33.1) 9 (15.5) 49 (27.4)	35 (28.9) 2 (3.4) 37 (20.7)	4 (3.3) 1 (1.7) 5 (2.8)	0 1 (1.7) 1 (0.6)	1 (0.8) 2 (3.4) 3 (1.7)	0 1 (1.7) 1 (0.6)
AGC/ SIL	Pos 41 Neg 7 P+N 48	15 (36.6) 4 (57.1) 19 (39.6)	14 (34.2) 1 (14.3) 15 (31.3)	0 0 0	0 0 0	0 0 0	0 0 0
Total	Pos 529 Neg 1328 P+N 1857	157 (29.7) 153 (11.5) 310 (16.7)	89 (16.8) 8 (0.6) 97 (5.2)	28 (5.3) 2 (0.2) 30 (1.6)	1 (0.2) 21 (1.6) 22 (1.2)	8 (1.5) 50 (3.8) 58 (3.1)	1 (0.2) 4 (0.3) 5 (0.3)

Conclusions: This is the largest study of the follow-up on the cases with AGC/HPV testing. hrHPV testing in women with various types of AGC may be useful to predict the risk of cervical high grade squamous/glandular neoplasias, but has no significant value in evaluating the risk of endometrial carcinomas.

397 Microphthalmia Transcription Factor (MiTF) Immunohistochemistry for Fine Needle Aspiration Biopsy of Ocular Malignant Melanoma

Carmen Perrino, Jeff Wang, Brian Collins. Washington University, St. Louis, MO.

Background: MiTF is a sensitive and specific immunohistochemical (IHC) marker of melanocytic differentiation and has been characterized in dermal and ocular malignant melanoma (MM). Its use in cytology specimens has not been extensively studied. Fine needle aspiration (FNA) biopsy can be performed during radioactive plaque insertion for the treatment of clinically diagnosed ocular MM, and IHC can contribute to its evaluation and diagnosis.

Design: A search of the pathology database for ocular FNA biopsies diagnosed as MM or suspicious for MM from 1998 to 2012 was completed. All pathology reports were reviewed and IHC results were recorded. All slides were re-reviewed to confirm the diagnosis and to assess the amount of tissue present on alcohol fixed, Papanicolaou-stained direct smears. Slides with adequate cellularity were destained and MiTF IHC was performed. Any amount of nuclear staining was considered positive, and tumor staining was scored on a scale of 1 to 4 (1: <25%; 2: ³25-49%; 3: ≥50-74%; 4: ≥75%).

Results: 274 cases were diagnosed as MM and 24 cases as suspicious for MM. IHC stains were performed on cell blocks prepared from 25% (69/274) of MM and 33% (8/24) of suspicious for MM cases. In MM cases, 23 immunostains were used, with the most common being MelanA (77% [44/57] positive, 12% [7/57] negative, 11% [6/57] non-contributory), HMB-45 (90% [26/29] positive, 7% [2/29] negative, 3% [1/29] non-contributory), and cytokeratin (92% [36/39] negative, 8% [3/39] non-contributory). In suspicious cases, 9 immunostains were used, with the most common being MelanA (75% [6/8] positive, 25% [2/8] non-contributory) and cytokeratin (80% [4/5] negative, 20% [1/5] non-contributory). MiTF IHC on destained Papanicolaou slides was performed in 37% (101/274) of MM cases, of which 82% (83/101) were positive, 4% (4/101) negative, and 14% (14/101) non-contributory. Among positive cases, the majority had score 3 (16%, 13/83) or score 4 (77%, 64/83) staining. MiTF IHC on destained Papanicolaou smears was performed in 33% (8/24) of suspicious for MM cases, of which 38% (3/8) were positive and 62% (5/8) non-contributory. Among positive cases, all (100%, 3/3) had score 4 staining. Sensitivity was calculated based on MM cases, and was 95% for MiTF IHC on destained Papanicolaou smears in comparison to 86% for MelanA IHC on cell blocks.

Conclusions: MiTF IHC showed a higher sensitivity compared to MelanA in the diagnosis of ocular MM. MiTF is a useful stain in the evaluation of ocular MM, and can be used on direct alcohol-fixed aspirate smears when cell block is inadequate.

398 An Analysis of Human Papilloma Virus (HPV) Testing and Endocervical Component on Pap Tests Performed From the Same Vial

Kirsten Pierce, Heather Currens, Laura Tafe, Gregory Tsongalis, Vijayalakshmi Padmanabhan. Dartmouth-Hitchcock Medical Center, Lebanon, NH.

Background: It is well known that HPV has a predilection for infecting cells of transformation zones. The presence of endocervical cells on a Pap test (PT) is an indication that the cervical transformation zone has been sampled. Existing evidence has shown that earlier repeat testing of women lacking an endocervical component is of little value in further detecting disease, thus a sample with no endocervical cells is not necessarily considered an inadequate sample. Both HPV testing and PT can be performed using a single sample; however, studies have not investigated the relationship between HPV results and the sampling of the transformation zone.

Design: Cervical/endocervical specimens were collected following the ThinPrep®liquid based PT platform. Specimens were submitted for either HPV reflex testing or concurrently with gyn cytology specimens. Cytology specimens were processed using the Hologic Cytyc T-3000 processor and ThinPrep® Imaging System. HPV testing was performed using the Roche Cobas ® HPV test on post aliquoted specimens from the PT vial. Data was collected retrospectively from the laboratory information system on 200 patients who had received pap tests over a 2 month period. We collected 100 cases with and 100 cases without an endocervical component on PT. To maintain uniformity among cohorts, we included only cases diagnosed as negative for intraepithilelial lesion or malignancy. We compared this with HPV test results within each category.

Results: Six of the 100 cases without endocervical component and four of the 100 cases with endocervical cells present were positive for high-risk HPV (See Table).

	HPV +	HPV -
Endocervical Cells Present	4	96
Endocervical Cells Absent	6	94

Conclusions: Our study did not show a significant correlation between HPV testing results and endocervical component presence on Pap tests. However, our study was small and limited to cases that were negative for a pathologic diagnosis. Current guidelines recommend co-testing every 5 years with cervical cytology and HPV testing in women aged 30-65. The results of our study suggest that absence of an endocervical component on Pap test does not necessarily warrant re-testing for HPV infection.

399 Role of Non-HPV 16 and 18 Types in Cervical High Grade Neoplasia

Raghavendra Pillappa, Brock Neil, Michael Bugg. University of Tennessee Health Science Center, Memphis, TN; American Esoteric Laboratories, Memphis, TN; Trumbull Labs, Germantown, TN.

Background: HPV 16 and 18 account for roughly 70% of all cervical cancers worldwide. Recently FDA approved Cobas HPV DNA test as first line cervical cancer screening tool for women 25 and older. Women tested positive for HPV 16 or 18 are referred for colposcopy while other hrHPV positivity would trigger a pap test. Limited data is available and significant confusion exists as to the role of other hrHPV types and the management strategy.

Design: A Retrospective specimen bank search was performed to retrieve 338 high grade squamous intraepithelial lesions on pap cytology between August 2013 to April 2014 and were reviewed for their HPV status. The cobas HPV DNA testing method was used to detect hrHPV from residual PreservCyt solution cervical samples. The master mix reagent contains primer pairs and probes specific for 14 high-risk types and beta-globin DNA. Cobas HPV test resultswere available in 129 patients. The 129 patient resultswere further evaluated to determine the HPV prevalence types. Available clinical information, HPV genotyping result and histopathologic follow-up results were reviewed.

Results: Among a total 129 valid HSIL pap/HPV+ cases, 45 (35%) were positive for HPV 16 or HPV16 and other hrHPV types, 10 (7%) were positive for HPV18 or HPV 18 and other hrHPV types while 61 (47%) were other hrHPV positive but negative for both HPV 16 and 18, last 13 (10% were negative for all high risk genotypes. Co-

infection were present in 26 (20%) out of 129 cases, of which 19 (73%) cases were due to HPV 16 and other hrHPV and 7 (27%) cases were due to HPV 18 and other hrHPV types. Age of detection in the other hrHPV types range from 61% (less than 25 years group), 47% (25-30 years group) and 43% (more than 30 years group). 36 out of 129 women (27%) had documented histopathologic follow-up. Out of 36 histopathologic HSIL (CIN 2/3) , 12 cases (33%) were due to HPV16 or HPV 16 and other hrHPV, 3 cases (8%) were due to HPV18 or HPV 18 and other hrHPV and 9 cases (25%) were due to other hrHPV types.

Conclusions: Our data demonstrate that non HPV 16/18 genotypes were the most common in high grade cervical lesions in our patient population across all age groups. This data indicates that hrHPV genotypes other than HPV 16/18 can be a cause of HSIL and physician need to monitor all hrHPV genotypes.

400 Clinical Significance of Atypical Glandular Cells on Pap Test: Analysis of Over 3000 Cases of AGC Pap in a Large Academic Hospital

Dinesh Pradhan, Stell Patadji, Chengquan Zhao. University of Pittsburgh Medical Center, Pittsburgh, PA.

Background: AGC Pap interpretation and screening for glandular neoplasia remains a major challenge.

Design: A computer-based search was carried out on our pathology database to retrieve ThinPrep Pap tests with AGC interpretation over a 6 year span, from January 2008 to December 2013. The cases with histologic follow-up within one year after the AGC Pap were included.

Results: Of 589,830 Pap tests during the study period, 3709 (0.6%) cases were reported as AGC which included AGC-NOS (52.4%), AEC (28.0%), AMC (6.7%), AGC-FN (0.9%), AGC/ASC-H (7.5%), AGC/LSIL (2.2%) and AGC/HSIL (2.7%).3007 cases (81.1%) had histologic follow-up within one year after AGC Pap diagnosis. The average initial follow-up period was 1.6 months and average age of these women was 44.3 years. Histological follow-up results varied significantly among the AGC subtypes.CIN2/3 was identified in 5.6% cases and cervical AIS/ADC was identified in 1.9% cases. Endometrial carcinoma was found in 5.5% cases. When the cases were divided into different age groups, CIN2/3 was the most common high grade lesion in patients<30 years (10.1%), and in patients 30-49 years (6.5%). Endometrial carcinoma was the most common neoplasia in women ≥50 years (13.7%).

AGC Types	Case	CIN1 (%)	CIN2/3 (%)	AIS/ ADC (%)	EM Hyperplasia (%)	EM ca (%)	Metastatic ca (%)
AGC-NOS	1630	196 (12.0)	35 (2.2)	22 (1.4)	14 (0.9)	95 (5.8)	11 (0.7)
AEC	760	126 (16.6)	21 (2.8)	15 (2.0)	4 (0.5)	3 (0.4)	0
AMC	211	9 (4.3)	0	1 (0.5)	16 (7.6)	47 (22.3)	1 (0.5)
AGC-FN	26	0	3 (11.5)	8 (30.8)	0	12 (46.2)	1 (3.8)
AGC/ASC-H	227	63 (27.8)	49 (21.6)	8 (3.5)	2 (0.9)	6 (2.6)	1 (0.4)
AGC/LSIL	66	31 (47.0)	2 (3.0)	1 (1.5)	0	1 (1.5)	0
AGC/HSIL	87	17 (19.5)	59 (67.8)	2 (2.3)	0	2 (2.3)	0
Total	3007	442 (14.7)	169 (5.6)	57* (1.9)	36 (1.2)	166 (5.5)	14 (0.5)

*including 19 invasive adenocarcinoma

Conclusions: This is one of the largest AGC follow-up studies till date. AGC subtype and age significantly affect probability of follow-up results. 94.5% AGC-detected carcinomas were endometrial or metastatic, cancer screening using HPV alone may miss the detection of these cancers.

401 Epidermal Growth Factor (EGFR) Mutational Genotyping Using Next Generation Sequencing on Non-Small Cell Lung Carcinoma (NSCLC) Using Liquid Based Cytology Samples

Jordan Reynolds, Zhen Wang, Yaxia Zhang, Eugen Minca, Maureen Jakubowski, Jennifer Brainard, Roger Klein, Carol Farver, Francisco Almeida, Yu-Wei Cheng. Cleveland Clinic, Cleveland, OH.

Background: Detection of *EGFR* gene mutations of NSCLC on residual cell pellets derived from liquid based cytology (LBC) samples has been validated using allele specific polymerase chain reaction (ASPCR) at our institution. The aim of this study is to validate next-generation sequencing (NGS) technology for detecting gene mutations on the residual cell pellets from the LBC container.

Design: Archived extracted DNA from LBC samples in PreservCyt of adenocarcinoma, with known EGFR mutational status, was retrieved. The samples' genomic DNAs were extracted, multiplex amplified, and enriched using Ion AmpliSeq Cancer Hotspot panel v2 (CHPv2) chemistry and OneTouch2 instrument, followed by semi-conductor sequencing on Ion PGM platform (LifeTechnologies). The mutation hotspots of *BRAF, EGFR, ERBB2, FGFR1, KRAS, MET, and PIK3CA* genes were further analyzed using NextGENe and Torrent Suite bioinformatics tools. The resulted variants were correlated with the EGFR mutation data in the same specimen, which was previously assayed using the rotor-gene Q (RGQ)ASPCR method (Qiagen).

Results: Ten cases were retrieved from 2013-2014. Archived extracted DNA from LBC specimens were obtained in eight cases signed out as adenocarcinoma and their subsequent *EGFR* results were retrieved. The specinem types included FNA from 8 lung via endobronchial fine needle aspiration, 1 chest wall FNA, and 1 pleural fluid. The average DNA concentration was 29.1 ng/ul (range 10.5-65.8 ng/ul). The mutations detected in EGFR were in perfect concordance between the two platforms and included four L858R mutations, three exon19 deletions, and one exon20 insertion. No disease-associated sequence changes were found in the mutation hotspots of other six genes mentioned above. For control values, DNA was extracted from LBC vials of a malignant melanoma metastatic to the lung, and small cell carcinoma of the lung. Both cases were wild type *EGFR* on NGS All other analyzed mutations were wild type.

Conclusions: *EGFR* mutational analysis using NGS can be successfully performed on residual cell pellets derived from LBC samples. In our series the cases previously underwent IHC evaluation of the cell block to determine tumor subtype, and ASPCR mutational analysis, yet there was still sufficient DNA remaining for NGS. The use of the residual sample in LBC should be further explored as an avenue for molecular diagnostics and would prevent second procedures for additional material.

402 Intradepartmental Inter-Pathologist Variation in Diagnosing Atypical and Suspicious Urine Cytology

Jordan Reynolds, Tarik Elsheikh. Cleveland Clinic, Cleveland, OH.

Background: There has been reported wide variation in the rate of diagnosing atypical urine cytology amongst different institutions, ranging from 1.9- 31%. To the best of our knowledge, no study to date has examined variability amongst cytopathologists (CPs) within the same department.

Design: All urine cytology diagnoses made by 12 board-certified CPs over a 39 month period (7/1/2011-10/1/2014) were retrieved. Diagnostic categories included negative for malignant cells (neg), atypical urothelial cells (AUC), suspicious for malignancy (susp), and positive for malignant cells (pos). Pathologists who signed less than 500 cases were excluded from the study. CP years of experience were grouped (1-3 year, 4-15 years, >15 years).

Results: A total of 22,744 cases from the Cleveland Clinic were signed out by 12 subspecialty boarded CPs (mean=1895, range 557-3562 cases). Overall departmental rate during this time frame was neg 81.9%, AUC 15.1%, susp 1.3% and pos 1.7%. The CPs had variable experience ranging from 2-26 years (mean 10.1), and there was wide variation in AUC diagnosis rate (range 7.9-27.9%, median 14%, inner quartile range 10.2-17.5%). Susp and pos diagnoses, on the other hand, showed no significant variation (ranges 0.5-1.9% and 1-2.3%, respectively). No statistical significance was found between years of experience and AUC rate.

Pathologist	Atypical (AUC)	Suspicious	Postiive for malignancy	Number of cases	Years of Experience
A	7.9	0.9	1.2	2073	1-3
B	9.1	1.7	2.0	3246	>15
C	9.5	0.5	2.2	3562	>15
D	12.2	1.3	1.5	827	4-15
E	12.7	0.6	1.5	1861	4-15
F	13.2	1.7	1.5	2522	4-15
G	14.9	1.6	2.2	1835	1-3
H	15.9	1.9	1.4	1873	>15
I	16.0	1.4	2.3	865	1-3
J	18.0	1.2	1.9	2152	4-15
K	24.2	1.3	2.0	557	1-3
L	27.9	1.2	1.0	1371	>15

Conclusions: Rate of AUC varies widely, even amongst CPs within the same department. Higher rates of AUC are not related to level of experience, which suggests that these diagnoses are more dependent on individual threshold for atypia. This study emphasizes the need for agreed upon strict criteria to be established for AUC category, which could help reduce rate and variability in atypical urine diagnoses.

403 Spleen Fine Needle Aspiration: A Review of 30 Cases

Valentina Robila, Cora Uram-Tuculescu, Ema Dragoescu. Virginia Commonwealth University Health System, Richmond, VA.

Background: Spleen fine needle aspiration (SFNA) is not routinely performed in the US. The literature on SFNA is limited to a few series and case reports. Therefore, cytopathologists have limited experience in interpreting SFNA. This study investigates our experience with SFNA, focusing on the clinico-radiologic context in which the procedure was performed and the added value by the cytologic interpretation.

Design: SFNAs performed at our institution during 2000-2014 were retrieved. Demographic data, clinical history, radiologic findings, cytologic interpretation, and surgical pathology follow-up were recorded.

Results: 30 SFNA in 27 patients were identified (F:M=14:13, mean age 47.2 yrs, range 12-82 yrs), performed by ultrasound [US] (22), CT (3), and endoscopic-guided US (5). The clinico-radiologic indications for SFNA are summarized in Table 1. A specific cytologic diagnosis was rendered in 28 cases (93.3%). The most common interpretation was normal splenic tissue (10). These cases were cellular with abundant small lymphocytes suggesting a hematologic process. One clue was the red pulp as cohesive clusters of epithelioid cells arranged in a syncytium or elongated, vessel-like structures. The second most common diagnosis was malignant lymphoma (ML). SFNA

allowed de novo diagnosis and precise classification (3 diffuse large B cell lymphoma, 2 Hodgkin lymphoma, 1 T-cell rich B-cell lymphoma). A rare case of sclerosing angiomatoid nodular transformation of the spleen (SANT) was encountered.

Clinico-radiologic indications	Cytologic interpretation	Follow-up
Solitary splenic lesions (14)	Abscess (4)	Cultures positive (2)
	Normal splenic tissue (3)	Splenectomy: fibrosis (2)
	Cyst (3)	
	ML (1)	
	Suspicious for ML (2)	
	Extramedullary hematopoiesis (1)	
	SANT (1)	Surgical excision: SANT (1)
Multiple splenic lesions (10)	Normal splenic tissue (3)	Splenectomy: lymphangioma (1)
	Granulomas (2)	Splenectomy: granulomas (1)
	ML (2)	
	Atypical (1)	Spleen biopsy: ML (1)
	Nondiagnostic, scant cellularity (2)	
Splenomegaly (3)	ML (2)	
	Normal splenic tissue (1)	
Accessory spleen (3)	Normal splenic tissue (3)	

Conclusions: The most common situation in the interpretation of SFNA is to recognize normal splenic tissue. This interpretation is useful, as our data indicates a benign follow-up. ML with specific subtyping and, rarely, primary splenic neoplasms can be diagnosed on SFNA. For these latter cases, ancillary studies are extremely useful in complementing the cytomorphology.

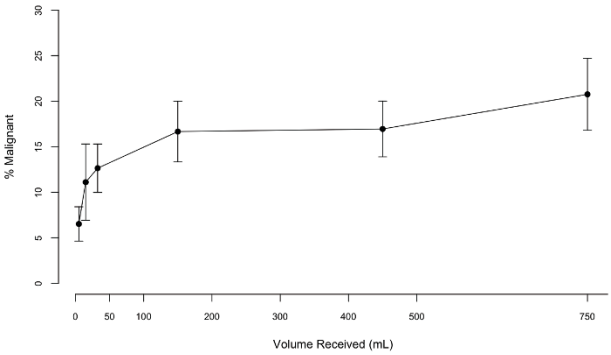
404 A Minimum Volume of 60 mL Is Necessary To Ensure That a Pericardial Effusion is Benign: A Retrospective Review of 480 Pericardiocentesis Specimens From 1994-2013

Lisa Rooper, Syed Ali, Matthew Olson. Johns Hopkins Hospital, Baltimore, MD.

Background: Although the importance of pericardial fluid cytology for identifying malignant pericardial effusions is well established, the role of specimen volume in cytologic diagnosis has never been explored. While any volume is sufficient for diagnosis if malignant cells are present, it is difficult to distinguish absence of disease from under-sampling of malignancy in small-volume effusions with benign findings. Recent studies have demonstrated that a minimum volume of 50-75 mL is necessary for diagnostic adequacy in pleural effusions, but this issue has never been investigated in pericardiocentesis specimens. We aim to determine the optimal volume of pericardial fluid necessary for cytopathological diagnosis.

Design: We identified all 480 pericardial fluid specimens with a documented numerical volume received at our institution between January 1994 and December 2013. Specimen and patient characteristics were tabulated for all cases. We then binned the specimens into six groups of roughly equivalent sample size and calculated the malignancy fraction (percentage of cases with cancer diagnoses) for each bin. These bins were combined at various cutoff points and evaluated using the Fisher exact test to determine an optimal minimal specimen volume.

Results: The pericardial effusion specimens had a median volume of 245 mL (range 1-2500 mL) with an overall malignancy fraction of 15.6%. As shown in Figure 1, the malignancy fraction increased from 6.5% for volumes less than 10 mL to 20.7% for volumes greater than 600 mL (p=0.03). The cumulative malignancy fraction in bins greater than or equal to a cutoff point of 60 mL was 18.1%, significantly larger than the 10.6% malignancy fraction of bins less than 60 mL (p=0.03).



Conclusions: These data demonstrate that fewer malignancies are detected in small volume pericardiocentesis specimens. As such, a volume of 60 mL or greater is necessary to minimize false-negative pericardial effusion cytology. Because the malignancy fraction continues to increase across all volume bins, we recommend all pericardial fluid not needed for other studies be submitted for cytologic evaluation.

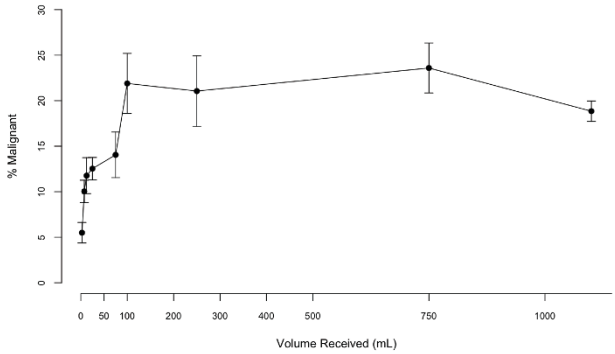
405 A Minimum Volume of 80 mL Is Necessary To Ensure That Ascites Fluid Is Benign: A Retrospective Review of 2,666 Cases From 1994-2013

Lisa Rooper, Syed Ali, Matthew Olson. Johns Hopkins Hospital, Baltimore, MD.

Background: Although ascites fluid is notorious for accumulating in massive volumes, the quantity submitted for cytologic evaluation can be quite small. Unfortunately, the role volume plays in cytologic diagnosis of paracentesis specimens has never been established. Obviously, malignant cells are diagnostic regardless of specimen volume. However, benign findings in small-volume samples could represent either true absence of disease or under-sampling of malignancy. Recent research has suggested that a minimum volume of 50-75 mL is necessary for diagnosis in pleural effusions, but no studies have previously evaluated adequate volume for ascites specimens. We aim to determine the minimum volume of fluid necessary for cytopathological diagnosis of malignancy in paracentesis specimens.

Design: We identified 2,666 paracentesis specimens with documented numeric volumes received at our institution between January 1994 and December 2013 and tabulated specimen and patient characteristics. We then binned the cases into nine groups of roughly equivalent sample size and calculated the malignancy fraction (percentage of cases with malignant diagnoses) for each group. Finally, we evaluated these bins using the Fisher exact test to determine an optimal minimal specimen volume.

Results: The paracentesis specimens had a median volume of 550 mL (range 1-10,000 mL) with an overall malignancy fraction of 16.8%. As shown in Figure 1, the malignancy fraction increased from 5.5% for specimens less than 5 mL to 21.8% for specimens 80-200 mL (p<0.001). Specimens with volumes greater than 200 mL had malignancy fractions that were independent of volume. The proportion of atypical and nondiagnostic specimens also decreased from 34.1% for specimens less than 5 mL to 8.2% for specimens greater than 1000 mL (p<0.001).



Conclusions: Significantly fewer malignancies are detected in small volume ascites specimens. These data suggest that submission of less than 80 mL of ascites fluid increases the risk of an indeterminate or false negative diagnosis. As such, a fluid volume of greater than 80 mL is necessary to minimize the influence of specimen size on diagnostic adequacy in paracentesis specimens.

406 The Evaluation of MicroRNAs Panel on Liquid Based Cytology of Thyroid Lesions – A Promising Role for miR-375 in a Preliminary Study

Esther Rossi, Tommaso Bizzarro, Maurizio Martini, Mirna Bilotta, Sara Capodimonti, Guido Fadda, Fernando Schmitt, William Faquin, Marc Pusztaszeri, Luigi Larocca. Catholic University, Rome, Italy; University of Luxembourg, Luxembourg, Luxembourg; Harvard University, Boston, MA; Geneva University, Geneva, Switzerland.

Background: Fine Needle aspiration cytology(FNAC) plays a major role for the evaluation of thyroid lesions. In spite of its high diagnostic accuracy,25% nodules are categorized as follicular neoplasms(FN) with varying risk of malignancy. Ancillary techniques,including microRNA(miRNA),are reshaping and empowering FNAC. MiRNAs are endogenous short single-stranded non-coding RNA wrking as negative regulators of gene expression. Over 1000human miRNAs have been identified and seemed to play an important role in tumor development. Scant data explored the role of miRNAs and the link with specific thryoid histotypes. In this preliminary study we investigated a panel of 5miRNAs in thyroid liquid based preparation (LBP).

Design: From January-April 2014, 13prospective FNs with histological control were enrolled. Eleven benign lesions (BL) and 11positive per malignancy (PM) were randomly selected as controls. The FNs histology resulted in 4malignancies (1Follicular Carcinoma-FC, 1Papillary thyroid carcinoma(PTC) and 2Follicular variant of PTC-FVPCS)) and 9Follicular adenomas(FA). Our 11PMs included 6FVPCs,3PTCs (including 2 microPTC), 2Medullary Carcinomas (MTCs). MiRNA was extracted from LBP and quantified by real time PCR. The expression of 5miRNAS(10b, 92a, 221, 222, 375)was analyzed.

Results: MiR-375 resulted in 8.2fold change over-expression in the FNs category vs BLs(p=0.045) whilst in 18.9 comparing PMs vs BLs(p=0.048). The use of a cutoff of 10 miR-375/miR-U6 relative ratio recognized 100%BLs, 36%FNs(4FA) and 18%PMs (2microPTC). Among the remaining 7FNs, the 2FVPCs showed higher values than PTCs. Among the 9PMs, the value increased significantly from PTCs to 6FVPCs up to 2MTCs. We did not find statistical significance in the other MicroRNAs.

Conclusions: LBP is a suitable method for the evaluation of microRNAs. MiR-375, over-expressed in 64%FNs and 82% PMs (including MTCs) represents a valid marker in ruling out BL and appraising FVPCs. Its over-expression may support its proliferative role targeting the known trascriptional coactivator yap-1(growth inhibitor) and contribuiting to the molecular mechanism of thyroid carcinogenesis.

407 The Role of Monocarboxylate Transporters in the New Insights of Morphological Features of BRAFV600E Mutation – A Preliminary Study

Esther Rossi, Tommaso Bizzarro, Maurizio Martini, Emilia Luca, Guido Fadda, Fatima Baltazar, Fernando Schmitt, Luigi Larocca. Catholic University, Rome, Italy; Minho University, Braga, Portugal; Luxembourg University, Luxembourg, Luxembourg.

Background: *BRAF^{V600E}* mutation, usually performed by DNA techniques, is the most common diagnostic/prognostic marker in papillary thyroid carcinoma (PTC). Only recently few papers have proposed the morphological prediction of *BRAF^{V600E}* on PTCs. The cytological evaluation revealed plump cells (eosinophilic cytoplasm and PTC nuclei) and peculiar sickle nuclear shape. These features may be attributed to a glycolytic phenotype. Monocarboxylate transporters 1-4 (MCTs) play a main role in transporting lactate and other monocarboxylates across membranes coupled with a proton. Herein, we investigated the correlation between these morphological features and MCTs.

Design: We analyzed 19 cyto-histological samples diagnosed as "positive for malignancy-favoring PTC" on LBC between January 2012-December 2013. All cases underwent mutational analysis (Pyrosequencing, Diatech-Italy) for *BRAF^{V600E}* and MCTs immunostaining (Temecula, CA) on LBC and histology. MCTs were scored semi-quantitatively for cellular membrane from 0 to 3+ (strong positivity) based on both the intensity of expression and its distribution in the tumor cells.

Results: Our 10 mutated and 9 wild type (WT) cases showed 100% cyto-histological concordance. The cytological evaluation revealed plump cells and sickle nuclear shape in 100% mutated cases. MCT1 yielded 70% positivity in the mutated cases especially in both the plump cells and sickle shape nuclei whereas the 9 WT cases resulted in negativity for MCT1 and absence of plump cells and sickle shape nuclei (p=0.048). The analysis of the other MCTs did not highlight any statistical significance in the two analyzed groups.

Conclusions: This is the first report of MCTs characterization in PTC with morphological *BRAF^{V600E}* features. This MCT1 expression may justify the plump characteristics of these *BRAF^{V600E}* PTCs due to lactate accumulation.

408 Factors Affecting the Success of Next-Generation Sequencing in Cytology Specimens

Sinchita Roy Chowdhuri, Rashmi Goswami, Hui Chen, Bedia Barkoh, Jawad Manekia, Keyur Patel, Mark Routbort, Rajesh Singh, Russell Broaddus, L Jeffery Medeiros, Gregg Staerkel, Rajyalakshmi Luthra, John Stewart. University of Texas MD Anderson Cancer Center, Houston, TX.

Background: Next-generation sequencing (NGS) is particularly challenging in cytology samples due to the limited nature of most samples. The aim of this study was to review cytology cases sent for mutational analysis to identify factors that affect success of NGS.

Design: A retrospective study of 141 consecutive cytology specimens reviewed by a cytopathologist, meeting adequacy criteria of >20% tumor cells, were analyzed by NGS. DNA was extracted from circled tumor-rich areas of formalin-fixed paraffin-embedded (FFPE) cell block unstained sections (USS) as well as direct smears (Diff-Quik and Papanicolaou stained). The cases were analyzed by NGS using the Ion Torrent PGM (Life Technologies, CA) and the Ampliseq Cancer Hotspot v2 panel.

Results: 102 of 141 cases (72%) were successfully sequenced by NGS. Cell block sections were available on 73 cases and smears were used for 68 cases. The median estimated tumor percentage and DNA concentration was 80% (range 20-100%) and 1.5ng/ul (range 0-32.5ng/ul) respectively. 39 of 141 cases (28%) failed NGS (quantity not sufficient (QNS)) either due to low DNA yield or failed template/library preparation. The median estimated tumor percentage and DNA concentration for the QNS cases was 60% (range 20-100%) and 0.2 ng/ul (range 0-23.2 ng/ul) respectively. 13 of 39 QNS cases (33%) had concurrent core needle biopsies (CNB) that were deemed inadequate/inferior for NGS testing, while the remaining 26 cases did not have a concurrent CNB (pleural fluids (n=2), fine needle aspirations of deep organs (n=9) and endobronchial ultrasound (EBUS) guided lymph nodes (n=15)). Of the 39 QNS cases, 8 were not run on an orthogonal platform as no specific gene was requested. The remaining 31 cases were sequenced on an alternate platform (Sanger/pyrosequencing) and 25 cases (81%) had mutation results for all requested genes, whereas 6 cases had partial results (failure/suboptimal sequencing for 1 or more genes). Multiple factors were identified that affect NGS success including specimen preparation, type of slide, tumor cellularity, pathologist bias (number and type of slides selected), DNA yield, and input DNA. A pilot quality improvement study for NGS analysis regardless of DNA concentration (not the manufacturer's recommended >0.85ng/ul; 10ng) showed a marked increase in NGS success rates from 59% (45/76) to 88% (57/65).

Conclusions: NGS failure in cytology samples is frequently a result of suboptimal DNA due to multiple preanalytical factors. Recognizing these factors can provide for better selection of cytology specimens for mutation analysis.

409 Effect of Multiple Human Papilloma Virus (HPV) Infections in High Grade Squamous Intraepithelial Lesions (HSIL)

Katrina Salazar, Haijun Zhou, Mary Schwartz, Dina Mody, Yimin Ge. Houston Methodist Hospital, Houston, TX.

Background: High-risk human papilloma virus (HR-HPV) infection is a well-established cause of cervical cancer and precancerous lesions led by HPV 16 and 18. The pathogenic effect of HPV in cervical cancer has been mainly examined with individual genotypes of HR-HPV and the interactions between HPV genotypes are largely unknown in women with multiple HPV infections.

Design: HPV DNA was extracted from 743 residual SurePath specimens collected between December 1, 2009 and March 30, 2011. HPV genotypes were determined by DNA microarray and confirmed by DNA sequencing.

Results: HR-HPV infections were associated with a higher rate of high grade squamous intraepithelial lesions (HSIL; 12.8%) than low-risk HPV (LR-HPV; 2.8%). There was no significant difference between single HR-HPV infections (11.5%) versus multiple HR-HPV infections (12.8%) in cases with HSIL. The rates of HSIL in groups with infection of 1, 2, or at least 3 HR-HPV genotypes were 11%, 10% and 6%, respectively. The group with mixed HR-HPV and LR-HPV infections had an intermediate rate of HSIL (6.7%). Cases with HPV 16/18 infection alone had a high rate of HSIL of 15.7%, compared to 4.8% when co-infected with non-16/18 HR-HPV and to 3.2% when co-infected with LR-HPV.

Conclusions: HR-HPV infections were associated with a higher rate of HSIL than LR-HPV. The lower HSIL rates in cases with at least 3 HR-HPV genotypes or with mixed HR-HPV and LR-HPV genotypes raises a possibility of potential competitive effect between the HPV genotypes. This notion is further supported by the lower HSIL rates in cases with co-infection by HPV 16/18 with either non-16/18 HR-HPV or LR-HPV genotypes compared to HPV 16/18 alone. The findings are suggestive of a potential competitive interaction between some HPV genotypes in patients with multi-genotype infection.

410 Inflammatory Kidney Disease and Renal Cell Carcinoma Mimicking Atypical and Suspicious Urothelial Cells in Urine Cytology: A Retrospective Review With Correlation With Surgical Pathology

Khaled Sarah, Matthew Luke, Therese Bocklage. University of New Mexico School of Medicine, Albuquerque, NM.

Background: Non-neoplastic urothelial cells may show atypical or suspicious features in urine cytology. However, the incidence of these diagnoses due to kidney disease has not been systematically studied. Recently, expert cytopathologists have proposed dividing "atypical urothelial cells," into low risk and high risk categories. We sought to determine whether nephritis and renal carcinoma affected urine cytology diagnoses and compared the predictive value of two high risk subcategories of "atypical urothelial cells".

Design: From 1290 cytologies, we found 40 patients with metachronous atypical or suspicious urine cytologies and urogenital biopsies. We evaluated the urine specimens for 13 features correlating with creatinine and BUN levels when available within three months of urine cytology, and follow-up (minimum of one year). We stratified the atypical diagnoses into low risk ("atypical") and two types of high risk (our in-house "suspicious" and Johns Hopkins proposed "atypical urothelial cells of uncertain significance- cannot exclude high grade urothelial lesion" [AUC-H]).

Results: The "suspicious" and "AUC-H" categories showed a significant improved positive predictive value for carcinoma diagnosis within one year (86% and 75%, respectively) compared to "atypical" (30%). The two high risk categories overlapped significantly. Renal cell and urothelial carcinoma could not be discriminated in the samples, as these cateogories by definition comprise cases with few abnormal cells (rather than a "positive" urine cytology diagnosis). Nephritis caused a significant number of atypical diagnoses but almost exclusively in the "atypical" low risk category.

Re-Review Urine Cytology Diagnosis	Cytology Sample Number	Nephritis Surg Path Dx (%)	RCC Surg Path Dx (%)	Urothelial Surg Path CA Dx (%)	Prostate CA Surg Path Dx (%)
Normal	11	9 (82)	1 (9)	1 (9)	0 (0)
Atypical	34	22 (65)	3 (9)	7 (21)	2 (5)
Suspicious	7	1 (14)	0 (0)	6 (86)	0 (0)
AUC-H	4	1 (25)	0 (0)	3 (75)	0 (0)
Casts	16	13 (81)	1 (6)	0 (0)	2 (13)

Conclusions: Nephritis may cause a spurious diagnosis of abnormal urothelial cells in urine cytology; most commonly in the low risk category. The presence of casts can provide a clue to the nephritis diagnosis but does not entirely exclude a coinciding malignancy.

411 Prostatitis and Prostate Carcinoma Mimicking Atypical and Suspicious Urothelial Cells in Urine Cytology: A Nine Year Retrospective Review

Khaled Sarah, Matthew Luke, Therese Bocklage. University of New Mexico, Albuquerque, NM.

Background: Non-neoplastic urothelial cells may exhibit atypical or suspicious features in urine cytology. However, the incidence of these urine diagnoses rendered due to prostate disease has not been systematically studied. Recently, expert cytopathologists have recommended dividing "atypical urothelial cells" into low risk and high risk categories. We sought to determine whether prostatitis and prostate carcinoma significantly result in misdiagnoses in urine cytology and compared the positive predictive value of two high risk subcategories of "atypical urothelial cells".

Design: In a nine year period, 1290 atypical or suspicious urine cytology diagnoses coincided in 64 patients with metachronous prostate biopsies or resections. We evaluated the urine specimens for 12 cytologic features and correlated results with UroVysion and serum PSA levels within three months of urine cytology (when available), and follow-up. We stratified the atypical diagnoses into low risk ("atypical") and two types of high risk (our in-house "suspicious" category and Johns Hopkins proposed "atypical urothelial cells of uncertain significance- cannot exclude high grade urothelial lesion" [AUC-H]).

Results: The "suspicious" and "AUC-H" categories showed a statistically significant improved positive predictive value for carcinoma diagnosis within one year (86% and 70%, respectively) compared to "atypical". These two categories overlapped significantly. Prostate and urothelial carcinoma could not be specifically distinguished from each other in the urine, because only a few abnormal cells were present (compared to a "positive" urine cytology). Prostatitis caused a significant number of atypical diagnoses but almost exclusively in the "atypical" low risk category.

Re-Review Urine Cytology Dx	Cytology Sample Number	Benign Surg Path Dx (%)	Prostatitis Surg Path Dx (%)	Prostate Ca Dx (%)	Urothelial Ca Dx (%)	Combined Prostate-Urothelial Dx (%)
Atypical	35	8 (23)	10 (29)	6 (14)	10 (29)	1 (3)
Suspicious	7	1 (14)	0 (0)	1 (14)	5 (72)	0 (0)
AUC-H	10	2 (20)	1 (10)	2 (20)	5 (50)	0 (0)

Conclusions: Prostate disease can shed cells misinterpreted as atypical or suspicious urothelial cells. Prostatitis seldom is mistaken for high risk urothelial cells. However, prostatic carcinoma cells can be interpreted as high risk urothelial cells using two high risk categorizing systems.

412 Stratified Mucin-Producing Intraepithelial Lesion (SMILE): An Uncommon Lesion as Source of Diagnostic Discordance With Potential Implications for Patient Management

Joerg Schwock, Marjan Rouzbahman, Matthew Cesari, Valerie Dube, Scott Boerner, Zeina Ghorab, William Geddie. University of Toronto, Toronto, ON, Canada; University Health Network, Toronto, ON, Canada; Sunnybrook Health Sciences Centre, Toronto, ON, Canada.

Background: SMILE is an uncommon HPV-related premalignant lesion of the uterine cervix thought to arise from reserve cells of the transformation zone. SMILE is frequently associated with concurrent high-grade squamous intraepithelial lesion (HSIL) and adenocarcinoma *in-situ* (AIS). Literature on the histopathologic features of the lesion and clinical management implications is limited. Exploration of the impact of SMILE on detection and classification of preinvasive lesions in cytology has so far been limited to few individual cases.

Design: We performed natural language searches of the laboratory information systems of two tertiary care centers for the years 2000 to 2014 using combinations of words and acronyms that included "SMILE", "adenocarcinoma", "adenosquamous", "stratified" and "in-situ". Resection specimen and immediately preceding Pap test were retrieved for each case in which the report suggested the presence of an *in-situ* lesion with features of SMILE without concurrent invasive carcinoma. Resections were reviewed and grouped according to the estimated proportion of SMILE (≥50% *versus* <50%), HSIL and AIS. Pap tests were reviewed by three expert cytopathologists (WRG, ZG, VD) blinded to the proportions determined by histologic review.

Results: A total of 13 cases of SMILE were identified. Median patient age was 33 years; range 23-51. SMILE was the dominant lesion (≥50%) in 6 cases. AIS was the dominant lesion in 1 case. HSIL and AIS were found in the same resection in 10/13 (77%) and 8/13 (62%) cases, respectively. Significantly more discrepancies among initial and review cytologic diagnoses were found in the SMILE-dominant group (p=0.029138; Fisher exact test). The initial diagnoses in this group were HSIL (n=2), atypical glandular cells (n=1), atypical glandular cells with ASC-H (n=1), AIS and LSIL (n=1) and LSIL with reactive endocervical cells (n=1). The single case with dominant AIS was diagnosed as NILM with reactive changes. All 6 HSIL-dominant cases were correctly identified by cytology. A positive or suspicious margin of the resection specimen was reported in 5/6 SMILE-dominant *versus* 3/7 HSIL/AIS-dominant cases.

Conclusions: SMILE is a lesion to consider in the cytologic differential diagnosis of reactive or atypical glandular abnormalities and in cases resembling HSIL with endocervical gland involvement.

413 TROP-2: Potential Diagnostic Utility for Identifying Papillary Thyroid Carcinoma

Anthony Simms, Cynthia Cohen, Momin Siddiqui. Emory University Hospital, Atlanta, GA.

Background: TROP-2 is a type I transmembrane glycoprotein reported to be overexpressed in various carcinomas, including papillary thyroid carcinoma (PTC). In contrast, TROP-2 is expressed in little to no adult somatic tissue. TROP-2 is related to EpCAM also called TROP-1, gp40 and KSA. TROP-2 staining of thyroid fine needle aspiration (FNA) samples has not been previously reported. In this study, we evaluated PTC FNA cell blocks (CB) for TROP-2 expression and compared them to other thyroid benign, atypical, neoplastic and malignant diagnoses.

Design: Immunohistochemical (IHC) evaluation for TROP-2 was performed on 80 thyroid FNA CB in paraffin sections of PTC (64), anaplastic thyroid carcinoma (2), follicular neoplasms (8), atypical (2), and benign cases (4). The staining was graded as positive or negative. Tumor cell membranous staining of 5% in the CB sample was considered positive.

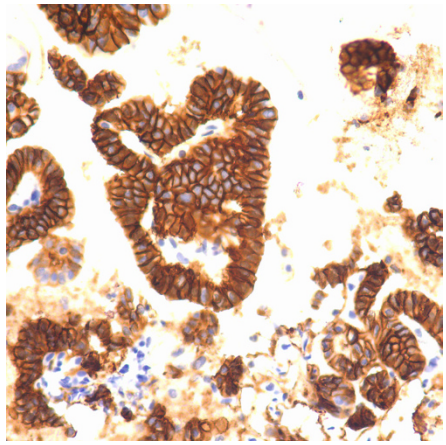
Results: Table 1: TROP-2 staining in 80 thyroid FNA CB

Diagnosis	Total Positive Cases (%)
PTC (n=64)	61 (95%)
Anaplastic thyroid carcinoma (n=2)	0
Follicular neoplasms (n=8)	0
Atypical cells (n=2)	0
Benign (goiter) (n=4)	0

Table 2: Statistical analysis of TROP-2 expression in PTC.

Diagnosis	Sensitivity	Specificity	PPV	NPV
PTC	95.31%	100%	100%	82.35%

Figure 1: TROP-2 IHC exhibiting membranous staining of PTC.



Conclusions: TROP-2 is a highly sensitive and specific IHC marker for identifying PTC. It has a high PPV and NPV for identifying PTC. TROP-2 may play a very important role in diagnosing PTC, especially in equivocal cases, and cases with a limited cellular sample in FNA CB's. Additionally, it may be a very helpful marker for confirming a diagnosis of PTC and separating it from benign follicular cells.

414 Cytologic Categorization of Pancreatic Neoplastic Mucinous Cysts: A Retrospective Study Based on the Papanicolaou Society of Cytopathology Guidelines

Amber Smith, Fadi Abdul-Karim, Abha Goyal. Cleveland Clinic, Cleveland, OH.

Background: Cytology plays a pivotal role in the preoperative diagnosis of pancreatic cysts. Here we have employed the Papanicolaou Society of Cytopathology (Pap Society) guidelines to reclassify and to assess the malignancy risk of cytology diagnoses of histologically proven neoplastic pancreatic mucinous cysts.

Design: A retrospective database search (January 2000 - June 2014) was performed for pancreatic neoplastic mucinous cyst resections with fine needle aspiration within the year preceding the resection. Histologic diagnoses were reclassified according to the 2010 WHO criteria. Cytologic, biochemical and imaging findings were recorded. For "atypical" or "suspicious" diagnoses, the FNA slides were reviewed blinded to histologic diagnoses. The cysts were reclassified according to the Pap Society guidelines and the findings were correlated with histology and imaging.

Results: 139 cases of pancreatic neoplastic mucinous cysts met the study criteria. 11 cases with atypical/suspicious cytology with unavailable slides were excluded. The remaining 128 cases included 81 intraductal papillary mucinous neoplasms, 47 mucinous cystic neoplasms. 29 cases were malignant - 17 with high grade dysplasia, 12 with invasive carcinoma. 86 cases had known carcinoembryonic antigen levels, 59 (67%) were >192 ng/ml. Cytologic reclassification with histologic correlation and malignancy risk is depicted.

Cytologic Diagnostic Category	Histologic Follow-up				Absolute Malignancy Risk
	Low grade dysplasia	Intermediate grade dysplasia	High grade dysplasia	Adenocar-cinoma	
Non-diagnostic (n=22)	14	4	0	4	18%
Negative (n=9)	6	3	0	0	0
Neoplastic: Other (n=78)	56	12	8	2	13%
Neoplastic with High-grade Atypia (n=11)	3	1	6	1	64%
Suspicious for Adenocarcinoma (n=5)	0	1	2	2	80%
Positive for Adenocarcinoma (n=3)	0	0	0	3	100%

Sensitivity of cytology for diagnosis of neoplastic mucinous cyst was 76%. Sensitivity, specificity, negative predictive value, positive predictive value and accuracy of cytology for diagnosis of malignancy (high-grade dysplasia or worse) were 50%, 95%, 87%, 74% and 79% respectively.

Conclusions: Our study reveals that Pap Society guidelines allow for accurate categorization of pancreatic neoplastic mucinous cysts on cytology with integration of ancillary data. The diagnostic categories (from negative to positive) are associated with an increasing risk of malignancy, aiding in patient risk stratification.

415 Precursor T-Lymphoblastic Lymphoma: Cytomorphological and Immunophenotypic Spectrum in Fine Needle Aspiration and Effusion Cytology Samples

Radhika Srinivasan, Poonam Bhaker, Upasana Gautam, Amita Trehan, Deepak Bansal, Pankaj Malhotra, Gaurav Prakash, Arvind Rajwanshi. Postgraduate Institute of Medical Education and Research, Chandigarh, India.

Background: Precursor T lymphoblastic lymphoma (Pre-T-LBL)/ leukemia is a neoplasm of lymphoblasts committed to T cell Lineage. A rapid diagnosis is essential in these patients who may present with respiratory distress. Fine needle aspiration cytology (FNAC) with confirmation by flow cytometric immunophenotyping (FCI) provides a quick, reliable diagnosis aiding early institution of chemotherapy. The aim of the present study was to report the cytomorphological spectrum of and flow cytometric immunophenotypic profile of Pre-T-LBL.

Design: A 3-year retrospective analysis (2011-13) was performed on all cases diagnosed as Pre-T-LBL on cytological samples. Giemsa and hematoxyline-eosin stained smears were evaluated. FCI and cell block immunohistochemistry (CB-IHC) was performed on the cell sediment obtained from FNAC / effusion cytology applying the lymphoma panel. Finally a detailed analysis of clinical, cytomorphology and immunophenotypic data was performed.

Results: A total of 15 cases of Pre-T-LBL (10 FNA and 5 pleural/pericardial fluid) were evaluated constituting 1.4% of all lymphomas diagnosed on cytological specimen during the study period. ***Cytomorphology:*** Smears showed sheets of small to intermediate sized lymphoblasts, with high nucleo-cytoplasmic ratio and scanty basophilic cytoplasm. Nuclear chromatin was fine and evenly dispersed. Nucleolus was inconspicuous in most cases (11) whereas 4 cases had 1-2 nucleoli in the larger blasts. Nuclear clefting was frequent; hand mirror shaped cells were seen in 9 cases. Mitotic count was high (8-30/10HPF). ***Immunophenotyping:*** Flow cytometry showed positivity for cytoplasmic and surface CD3 and CD2 in all cases; dim surface CD3 positivity was seen in 1 case only. Dual CD4/CD8 positivity was observed in all cases forming a tight cluster. Other markers expressed were CD5 (13/15), CD10 (7/15) and TdT (9/15) cases. None of the cases expressed CD34 and HLA-DR. The reactive B cell population varied from 0-20%. Overall, the immunophenotype was consistent with the cortical T-LBL subtype. CB-IHC showed the lymphoma cells to be uniformly CD3+/TdT+/CD20-.

Conclusions: Precursor T-Lymphoblastic Lymphoma has a characteristic morphology and immunophenotype enabling accurate and rapidly diagnosis on FNAC in combination with FCI.

416 Potential Impact on Rates of Malignancy for FNA Diagnostic Categories Were Non-Infiltrative, Non-Invasive Follicular Variant of Papillary Thyroid Carcinoma Not Termed Carcinoma

Kyle Strickland, Brooke Howitt, Jeffrey Krane, Justine Barletta. Brigham & Women's Hospital, Boston, MA.

Background: Increased recognition of the indolent nature of non-infiltrative, non-invasive follicular variant of papillary thyroid carcinoma (FVPTC) along with greater insight into the molecular alterations of these tumors has prompted endocrine pathologists to question whether these tumors warrant a diagnosis of carcinoma. However, a change in terminology would significantly affect the rate of malignancy of fine needle aspiration (FNA) diagnostic categories. The aim of this study was to determine the percentage decrease in associated risk of malignancy for each FNA diagnostic category were these tumors no longer termed carcinomas.

Design: We evaluated a cohort of 655 consecutive FNAs with subsequent resection specimens over a 22-month time period. The diagnoses of the preceding FNAs were recorded according to The Bethesda System for Reporting Thyroid Cytopathology. For cases with more than one preceding FNA, the FNA diagnosis associated with the highest risk of malignancy was identified. Slides for all resections with a diagnosis of FVPTC were reviewed to identify non-infiltrative, non-invasive tumors. By definition, all of these tumors were encapsulated, partially-encapsulated, or well-circumscribed and lacked any indication of infiltrative growth, capsular penetration, or lymphovascular invasion.

Results: Our cohort of 655 FNAs included 53 (8.1%) nondiagnostic (ND), 167 (25.5%) benign, 97 (14.8%) atypia/follicular lesion of undetermined significance (AUS/FLUS), 88 (13.4%) suspicious for follicular neoplasm (FOL), 94 (14.4%) suspicious for malignancy (SUS), and 156 (23.8%) malignant cases. Surgical resections demonstrated benign findings in 308 (47.0%) and malignant tumors in 347 (53.0%), including 85 non-infiltrative, non-invasive FVPTCs accounting for 24.5% of malignancies. Our rates of malignancy for ND, benign, AUS/FLUS, FOL, SUS, and malignant were 18.9%, 13.2%, 40.2%, 45.5%, 87.2%, and 98.7%, respectively. If non-infiltrative, non-invasive FVPTCs were no longer termed carcinomas, these rates would drop to 17.0% (10% decrease), 5.4% (59% decrease), 22.7% (44% decrease), 37.5% (18% decrease), 45.7% (48% decrease), and 93.6% (5% decrease), respectively.

Conclusions: Our findings demonstrate that if terminology were to change and non-infiltrative, non-invasive FVPTCs were not considered carcinomas, the rate of malignancy for FNA diagnostic categories would significantly decrease, with the most clinically significant change seen in the SUS category which demonstrated a decrease of nearly 50% in our cohort.

417 Endobronchial Ultrasound-Guided Fine Needle Aspiration (EBUS): An Effective Modality for Sampling Targeted Thoracic Lesions in Lung Transplant Recipients

Charles Sturgis, Jennifer Brainard, Sonali Sethi, Carol Farver, Marie Budev, Peter Mazzone, Fadi Abdul-Karim. Cleveland Clinic, Cleveland, OH.

Background: Lung transplantation (LTx) is performed for end-stage lung diseases which would be otherwise fatal. Five year survival after LTx is approximately 50%. Recipients are treated with immune supression and can experience rejection, infections, and malignancies. This study investigates the feasibility and diagnostic yield of endobronchial ultrasound guided fine needle aspiration (EBUS) in LTx patients with parenchymal lung lesions and thoracic lymphadenopathy.

Design: During 5 years from 05/09 to 05/14, 582 patients underwent LTx at Cleveland Clinic. A review of records indicated that 14 of these patients later underwent EBUS. Demographic and diagnostic parameters were recorded.

Results: Present in Table 1.

	Age (yrs)	Interval (mos)	Sex	Explant Diagnoses	Reason for EBUS	EBUS Sites	EBUS Diagnoses
1	68	25	M	UIP & emphysema	Lung mass & adenopathy	2L, 4L, 7, 10R	Negative
2	68	40	F	UIP	Lung mass & adenopathy	4R	Squamous carcinoma
3	54	19	F	UIP	Lung mass	Lung	Squamous carcinoma
4	62	18	M	UIP*	Lung mass & adenopathy	2R, 7	Squamous carcinoma
5	48	7	M	UIP	Adenopathy	4R	Small cell carcinoma
6	70	15	M	UIP*	Lung mass & adenopathy	2L, 4R, 7	Adenocarcinoma
7	67	10	F	UIP	Adenopathy	4L	Negative
8	67	5	M	UIP	Adenopathy	4R	Squamous carcinoma
9	67	11	M	UIP	Adenopathy	4R, 7	Negative
10	68	7	M	UIP & emphysema	Adenopathy	4R	B cell lymphoma (PTLD)
11	65	21	M	UIP	Lung mass	Lung	Sarcomatoid carcinoma
12	58	13	F	Emphysema*	Adenopathy	7, 11R, 11L, 12R	Negative
13	63	15	M	UIP	Lung mass & adenopathy	7, lung	Squamous carcinoma
14	69	6	F	Emphysema	Adenopathy	4R	Small cell carcinoma
	μ64	μ15		* Incidental carcinoma			

Conclusions: A total of 14 patients (μ age 64 years) underwent EBUS after LTx. The mean interval between LTx and EBUS was 15 months. EBUS yielded cytologic material diagnostic of malignancy in 10 (71%) of the patients with half of those being squamous carcinomas. EBUS can be performed safely in lung allograft recipients, and is of value in confirming and staging thoracic malignancies in this population. Carcinoma subtyping is feasible by EBUS, and ancillary studies to confirm clonality in post-transplant lymphoproliferative disorders are possible.

418 Comparison of the Diagnostic Utility of Electromagnetic Navigation Bronchoscopy (ENB) and CT-Guidance Biopsy in Evaluating Peripheral Lung lesions

Jihong Sun, Xiu Yang, Stephen Slone, Mostafa Fraig. University of Louisville Hospital, Louisville, KY.

Background: Peripheral lung lesions are beyond the reach of conventional bronchoscopies. Percutaneous CT-guided biopsies are often required for sampling. The Electromagnetic Navigation Bronchoscopy (ENB) is a new technique for peripheral lung lesions evaluation, utilizing an image localization system to direct a probe to the lung periphery. This technique has been used successfully in our institution for 2.5 years. We compare this technique with percutaneous CT-guided biopsy in diagnostic yield and procedure-related morbidities.

Design: Medical records were searched for ENB (ENB group) and percutaneous CT-guided (CT group) biopsies from 1/2012 to 8/2014. On-site evaluation by a pathologist was provided for all procedures. All adenocarcinoma cases were reflexed to molecular testing for EGFR mutation or ALK rearrangement. Data from radiographic imaging, clinical notes, pathology diagnosis, molecular testing, operational notes and post-procedure radiographs were reviewed.

Results: Search revealed 219 cases in ENB group, 96 men and 123 women, ages ranging from 18 to 91 years old (mean 57); 123 cases in CT group, 73 men and 50 women, ages ranging from 28 to 85 years old (mean 61). The pathologic diagnoses are listed in Table 1.

	ENB	CT-guidance
Total cases number	219	123
SCC	19	28
Adenocarcinoma	25	26
NSCLC	3	2
Neuroendocrine tumor	3	2
Metastatic cancer	5	22
Spindle cell tumor	0	6
Suspicious	3	1
Benign	159	33
Non-diagnostic	2	3

In ENB group, 25 cases were adenocarcinoma and 16 (64%) had sufficient tissue for molecular testing. In CT group, 26 cases were adenocarcinoma and 17 (65%) were sufficient. The non-diagnostic sampling rate is almost comparable in ENB group (1%) with CT group (2%). Compared to ENB group, CT group has significantly higher risk for pneumothorax requiring chest tube insertion (5.7% vs. 0%).

Conclusions: Our study demonstrates ENB is a safe modality in evaluating peripheral lung lesions. ENB performed at our institution had no incidence of pneumothorax. Meanwhile, ENB offers an equally adequate sampling for pathological diagnosis and molecular testing as CT-guided sampling. Further, ENB sampling is possible from trachea, lymph node, extending peripheral lung and can be combined with variety of methods- FNA, forceps biopsy, brushing and washing, which may increase the diagnostic yield.

419 Fine Needle Aspiration of the Kidney: A 22-Year Retrospective Single-Institution Analysis of 418 Cases

Matthew Swadley, Harvey Cramer, Robert Emerson, Shaoxiong Chen, Xiaoyan Wang. Indiana University School of Medicine, Indianapolis, IN.

Background: In recent years, utilization of renal fine needle aspiration (FNA) cytology has decreased due to concerns over potential procedural complications that include bleeding and needle-tract tumor seeding. In this study, we describe our institution's experience with renal FNA over a prolonged period and evaluate the diagnostic performance of this technique.

Design: All kidney FNA cases for the 22-year period between 1992 and 2014 were identified through a search of our department's LIS. All correlating surgical pathology reports of subsequent core biopsies and/or nephrectomy specimens were reviewed. For each case, the initial FNA diagnosis was compared to the final surgical pathologic diagnosis to determine the accuracy of renal FNA.

Results: Of our 418 renal FNA cases, 252 (60.3%) had subsequent or concurrent histopathologic evaluation. FNA was diagnostic of malignancy or neoplasm in 254 cases (60.7%), suspicious in 26 (6.2%), atypical in 32 (7.7%), benign (nonneoplastic) in 48 (11.5%) and unsatisfactory in 58 (13.9%). Malignant/neoplastic FNA diagnoses included renal cell carcinoma (154), oncocytic neoplasm (14), urothelial carcinoma (10), lymphoma (12), metastasis (19), malignant cells NOS (30), and other less common entities (15). Benign diagnoses included cyst contents, abscess, and inflammation. Twelve of 14 cases (85.7%) initially classified as "suspicious" and 16 of 20 cases (80%) initially classified as "atypical" by FNA were neoplastic on final histopathology. No false-positive FNA diagnoses were identified among 160 diagnostic FNA cases with surgical pathology follow-up. Two classification errors included a case diagnosed by FNA as poorly-differentiated carcinoma that proved to be metastatic melanoma and another FNA diagnosed as oncocytoma that was shown to be a renal cell carcinoma. None of the cases diagnosed as a nonneoplastic entity by FNA showed subsequent malignancy (20 cases with follow-up). Of 38 unsatisfactory FNA cases, 36 were found to be definitively neoplastic on subsequent biopsy or resection.

Conclusions: At our institution, renal FNA demonstrated a sensitivity of approximately 72% for neoplastic and benign entities with a specificity of 100% and with fewer than 2% of malignant FNA cases being incorrectly subclassified. Given its relatively high sensitivity and very high specificity, renal FNA remains a clinically valuable diagnostic tool.

420 Hematolymphoid Neoplasia of the Renal Parenchyma Diagnosed By Fine Needle Aspiration Cytology: A 22-Year Single Institution Retrospective Analysis

Matthew Swadley, Shaoxiong Chen, Robert Emerson, Harvey Cramer, Shanxiang Zhang, Jiehao Zhou, Xiaoyan Wang. Indiana University School of Medicine, Indianapolis, IN.

Background: Involvement of the renal parenchyma by a hematolymphoid neoplasm is an uncommon and often unexpected occurrence. Despite the proven utility and safety of fine needle aspiration (FNA) for deep solid organ sites, decreasing utilization of FNA biopsy of the kidney has been widely observed. In this study, we analyze our institutional experience of diagnosing hematolymphoid neoplasms involving the renal parenchyma by FNA.

Design: A computerized search of our LIS was performed for the 22-year period from 1992 to 2014 and all cases of hematolymphoid neoplasia involving the kidney diagnosed either by FNA and/or surgical pathology were identified. Review of these patients' medical records as well as FNA slides from these cases was performed.

Results: A total of 422 renal FNAs were evaluated at our institution and in 17 of these patients (4%), a diagnosis of a hematolymphoid neoplasia involving the kidney was established. The patients ranged in age from 9 to 89 years (mean 59.4) and were mostly male (10 of 17). In all, 7 patients had a known prior history of lymphoma or leukemia prior to the FNA biopsy. Review of the clinical records revealed that in 4 patients the hematolymphoid neoplasm was confined to the kidney. Flow cytometry had been obtained in 13 of the 17 FNA cases. The FNA diagnoses were positive in 12 (71%), atypical/suspicious in 2 (12%), and nondiagnostic in 3 (17%) due to a lack of lesional material. The 12 positive FNAs included 4 large B-cell lymphomas, 3 B-cell lymphomas NOS, 1 high-grade B-cell lymphoma, 1 follicular lymphoma, 1 low-grade B-cell lymphoma, 1 B lymphoblastic lymphoma/leukemia (B-ALL) and 1 T-cell lymphoma. Histological confirmation was obtained in the 12 positive FNAs revealing 4 diffuse large B-cell lymphomas (DLBCLs), 3 B-cell lymphomas NOS, 1 B-ALL, 1 Burkitt lymphoma, 1 follicular lymphoma, 1 low-grade B-cell lymphoma and 1 T-cell lymphoma. In addition, follow-up of 2 FNA cases diagnosed as atypical/suspicious showed 1 DLBCL, and 1 case that remained suspicious for lymphoma on histology. Follow-up histologic diagnoses for 3 nondiagnostic FNA cases included 2 DLBCLs and 1 B-ALL.

Conclusions: Hematolymphoid neoplasia involving the renal parenchyma is a rare occurrence, observed in only in 4% of our renal FNA cases. Most patients had no prior history of hematolymphoid neoplasia at the time of biopsy. When lesional material is available, FNA with rapid on-site evaluation coupled with flow cytometry is an accurate method of diagnosing hematolymphoid neoplasms involving the kidney.

421 Adrenal Gland (AG) Fine Needle Aspiration Cytology (FNAC): Analysis of 3886 Responses From the College of American Pathologists Non-Gynecological Cytopathology Education (CAP-NGC) Program

Z Laura Tabatabai, Guliz Barkan, Daniel Kurtycz, Rhona Souers, Rodolfo Laucirica, Diane Davey, Rosemary Tambouret. University of California, San Francisco, CA; Loyola University, Maywood, IL; University of Wisconsin, Madison, WI; College of American Pathologists, Northfield, IL; Baylor College of Medicine, Houston, TX; University of Central Florida, Orlando, FL; Harvard Medical School, Boston, MA.

Background: FNAC is a crucial method to determine if AG lesions represent metastatic or primary benign or malignant disease. This study was undertaken to determine the performance characteristics of AG FNAC by assessing participant responses in the CAP-NGC program.

Design: The analysis included 3886 responses based on 47 slides evaluated over a 6-year period and examined concordance to the general diagnosis for both malignant and benign cases. Two nonlinear mixed models were used to analyze the concordance rates.

Results: Malignant and benign cases included 2839 and 1047 responses, respectively. Cytotechnologists (CTs) performed significantly better than pathologists (PTs) (96.9% vs. 91.0%) for concordance to the general diagnosis of malignancy. Metastatic malignancy (MM) cases showed a significantly higher concordance rate than adrenocortical carcinoma (ACCA) cases for all types of participants (97.4% vs. 64.9%). CTs showed statistically better performance than PTs in diagnosis of ACCA (84.5% vs. 57.4%) and MM (98.8% vs. 96.4%). Pap-stained preparations performed significantly better than modified-Giemsa-stained preparations in malignant cases (93.7% vs. 86.0%). The most common discordant diagnosis for ACCA was benign adrenocortical cells/adenoma (BACC) (34.6%). MM cases had few false negative discordant diagnoses but were frequently misclassified as ACCA (19.5%). For benign cases, only the participant type was significantly associated with performance, with PTs performing significantly better than CTs (79.0% vs. 60.8%). The most common discordant reference diagnoses for benign cases were MM (9.6%), ACCA (7.9%), and non-hematopoietic small blue cell tumor (5.5%).

Conclusions: This study confirms that the greatest diagnostic challenge in AG FNAC is distinguishing ACCA from BACC. Pathologists may be reluctant to diagnose endocrine neoplasms as malignant without knowledge of clinical behavior, while cytotechnologists have a tendency to overcall AG lesions as MM and other malignancies. Education in recognition of BACC is suggested, and pathologist review of AG FNA cases may serve to compensate for cytotechnologist false positive interpretation.

422 History of High Risk HPV Testing and Pap Test Results in a Large Cohort of Patients With Cervical Carcinoma: Experience From the Largest Women Hospital in China

Xiang Tao, Zaibo Li, Xianrong Zhou, Zhiheng Wang, Yabin Yan, Chengquan Zhao. Obstetrics and Gynecology Hospital of Fudan University, Shanghai, China; Ohio State University Wexner Medical Center, Columbus, OH; Magee-Womens Hospital of University of Pittsburgh Medical Center, Pittsburgh, PA.

Background: In the 2012 ASCCP cervical cancer screening guidelines,co-testing with HPV and Pap tests every 5 years is preferred as primary screening method for women ages 30 to 65 years. FDA recently approved cobas HPV test alone for primary screening. Although, HPV testing is very sensitive for detecting cervical cancer precursor lesions, data of prior HPV history in cervical cancer patients are limited. In this study, we investigated prior hrHPV testing results in patients with invasive cervical cancer.

Design: We retrieved 274 patients [255 squamous cell carcinomas (SCC), 7 adenocarcinomas (AD), and 12 adenosquamous carcinomas (ADSCC)] with hrHPV testing results within 1 year before the histological diagnosis from January 2011 to June 2014. Pap test results within one year prior to histological diagnosis were also recorded. HC2 method was used for hrHPV testing.

Results: The average age of patients was 45.7 years (13-72). The average time period between hrHPV testing and histologic diagnosis was 28 days. HrHPV positive rate is 89% in all patients (89% in SCC, 71% in AD and 100% in ADSCC). 122 of the 274 (44.5%) patients had Pap test results and 80% of cases showed abnormal results. Four of 10 patients (40%) with negative hrHPV testing result had significant abnormal Pap results, including 2 cases of HSIL, 1 of AGC, and 1 of ASC-H.

Types	Cases	HPV Positive (%)	HPV negative (%)
Squamous cell carcinoma	255	227 (89.0%)	28 (11.0%)
Adenocarcinoma	7	5 (71.4%)	(2) 28.6%
Adenosquamous carcinoma	12	12 (100%)	0 (0%)
Total	274	244 (89.1%)	30 (10.9%)

Conclusions: Our data demonstrated 11% of patients with invasive cervical cancer had negative hrHPV results within one year before histologic diagnosis. Furthermore, the negative rate is much higher in cervical adenocarcinoma cases, suggesting some adenocarcinomas might be HPV-unrelated. 40% of HPV negative cancer cases had abnormal Pap results. Our data are challenging HPV testing alone to be used as a primary screening method. In addition, newly ASCCP recommended extended screening intervals (5 years for co-testing) should be further evaluated due to existence of invasive cancer cases with both negative Pap test and HPV testing.

423 Abstract Withdrawn

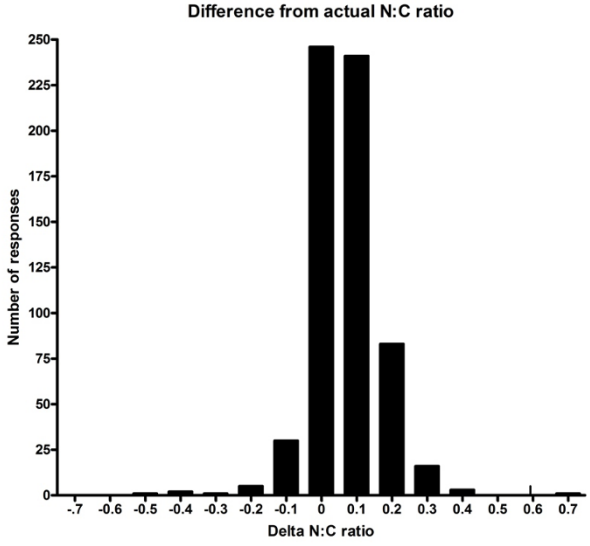
424 N:C Ratio Estimation: Just How Accurate Are We?

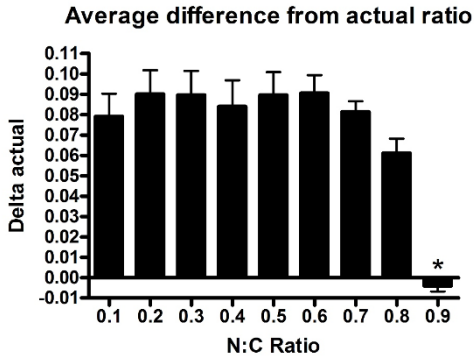
Louis Vaickus, Rosemary Tambouret. Massachusetts General Hospital, Boston, MA.

Background: Nuclear to cytoplasmic ratio (N:C ratio) is an important morphologic parameter in the assessment of atypia in histology, especially in cytopathology. Frequently, classification schemes recommend very specific, numerical cutoffs for an abnormally high N:C ratio (e.g 0.7). However, it's almost never specified how or if this ratio is measured. Rather, it is implied that we, as histopathologists, are intended to estimate this relationship (rather than using an ocular micrometer or other such measuring device). Therefore, we wanted to determine how accurate the N:C ratio estimates of various histomorphologists actually are.

Design: Graphical representations of cells with various N:C ratios (from 0.1 to 0.9) were created in MS Paint. Four basic schemes were employed for each ratio: centric nucleus, eccentric nucleus, circular cytoplasm and elliptical cytoplasm. N:C ratio was defined as the ratio of nuclear area to total cell area (cytoplasmic + nuclear area). Participants were sent a survey in Google Forms which asked them to select (from 12 options) an N:C ratio for each of 31 cell representations. The survey was sent to all AP and CP residents, and some attending pathologists and cytotechnologists. The results were compiled and statistics and graphs created in Graphpad Prism 4.0.

Results: The respondents' estimated N:C ratios were remarkably accurate. On average they differed from the actual N:C ratio by +0.079 (Std.Dev=0.10) (Figure 1). The mean difference from the depicted N:C ratio was relatively constant for ratios from 0.1 to 0.8. The mean difference from the actual ratio was statistically, significantly less for the highest N:C ratio depicted (0.9, $p < 0.01$)(Figure 2).





Conclusions: Trained histomorphologists are very accurate in their estimates of N:C ratios of graphical depictions of cells for a variety of cell shape and nuclear position. This data suggests that numerical cutoffs for determining atypical N:C ratios can be reliably estimated.

425 Endobronchial Ultrasound Guided Needle Aspiration Cytology(EBUS-TBNA) On-Site Evaluation(ROSE): Accuracy and Comparative Study Based on Lymph Nodes(LN) Sized<1.5cm and/or With Standarized Uptake Value (SUV)<4

Carmen Vasquez, Douglas Sanchez, Esther Pallisa, Mario Culebras, Margarita Alberola, Carmen Dinares, Carmela Iglesias, Irene Sansano, Santiago RamonyCajal, Natalia Tallada. Vall d'Hebron Hospital, Barcelona, Spain.

Background: The EBUS-TBNA applied to mediastinal LN is an advanced procedure in staging lung cancer tumors to define treatment protocols.

We report our 5-year experience with EBUS-TBNA-ROSE, cytological accuracy and histological correlation.An additional comparative study was performed on LN sized≤1.5cm and SUV≤4 with cytology.

Design: From 2010-2014, 97 patients were submitted to EBUS-TBNA-ROSE. Needle aspiration (22G) was applied on 137LN and 8mediastinal masses.The most frequent LN stations were 4R(34%) and 7(31%), others less than 10%.Cell material was evaluated on air-dried smears. PAP slides and cell block were obtained.Negative diagnosis required 3passes.

Results: From 97 patients: 66 had 1LN sampled; 23 patients/2; 5 patients/3 and 3 patients/4.LN size ranged from 0,7 to 6cm.From 137 LN samples,10(7%) cases were inadequate, 69(50%) negative, 4(3%) benign (granulomatous), 3(2%) suspicious for malignancy, and 51(38%) malignant diagnosis: adenocarcinoma(ADK)/21 cases; squamous cell cancer (SCC)/15; neuroendocrine carcinomas (NEC)/8; NSCC/ 5, urothelial metastasis/1 and lymphoma/1.Cytological results on 8 mediastinal lesions: 1 case (12.5%) insufficient; 1(12.5%) benign lesion (bronchogenic cyst), and 6(75%) malignant (ADK,1; SCC,1; NEC,3; and NSCC,1).In 50 patients the therapeutic decision was based only on cytologic results.Surgical histology confirmed 18 cases of cytologic diagnosis (negative and positive) on the same node/tumor.Correlation with surgical specimen type tumor revealed 14 positive LN and 1 benign granulomatous.Disaccord in type tumor in 1 case (ADK/SCC).Correlation failed in 9 cases due to the absence of the same LN previously sampled by EBUS.Inadequates:4.

Comparative study based on LN size≤1.5cm and/or SUV≤4 by PET disclosed: 66 LN applied one or both criteria and were subclassified in 3 groups in order to correlate with cytologic diagnosis.

a)Size≤1.5cm, 55 LN: 35(-); 10(+); 1 suspicious; 1 benign and 8 inadequate. b)SUV≤4, 32 LN: 25(-); 3(+); 1 suspicious and 3 inadequates. c)Size and SUV combined, 21 LN: 16(-); 1(+); 1 suspicious; and 3 inadequates.

Conclusions: Our accuracy is sensitivity (100%) and specificity (93%).

The comparative study disclosed a predominance of negative results.Positives results related to size(20%), SUV(12,5%) decreases significantly if both criteria are used(size and SUV,9.5%) being a good value to predict negative results.LN size is the less accurate criteria.However, further studies are needed to compare our results.

426 ThyroSeq v1 Next-Generation Sequencing (NGS) May Improve the Detection of Malignant Hurthle Cell Nodules

Madeline Vazquez, Seema Gollamudi, Samina Nur, Hossein Yazdi, Walter Blumenfeld, Ann Avitabile, Carlos Urmacher. CBLPath - A Sonic Healthcare Company, Rye Brook, NY.

Background: The term Hurthle Cell Nodule (HCN), usually classified as Bethesda III, describes follicular-derived nodules when Hurthle cells comprise more than 75% of the FNA. In the presence of background lymphocytes in chronic thyroiditis and abundant colloid in nodular goiter, a HCN can be classified as Bethesda II. A HCN with a microfollicular pattern can be classified as Bethesda IV. Malignancy can only be confirmed by the presence of capsular and/or vascular invasion. We report the results of ThyroSeq v1 NGS on HCNs to determine if it can improve the detection of malignancy pre-operatively.

Design: A prospective evaluation of 41 FNAs diagnosed as HCN underwent ThyroSeq v1 NGS with clinical and/or surgical follow-up. The ThyroSeq v1 can detect 284 cancer-associated mutations in 12 key thyroid cancer related genes (*NRAS, CTNNB1, PIK3CA, BRAF, RET, PTEN, HRAS, KRAS, TSRH, AKT, TP53* and *GNAS*). NGS was carried out on a Personal Genome Machine sequencer (Ion Torrent) using the Ion PGM Sequencing 200 Kit version 2.

Results: Of the 41 HCNs, there were 6 (14.28%) classified as Bethesda II; 4 (66.66%) had no mutations and 2 (33.33%) had *TSHR* mutations. There were 31 (75.6%) classified as Bethesda III; 21 (67.74%) had no mutations and 8 (25.8%) had single gene mutations. There was 1 (3.22%) case each of *BRAF*, *PAX8/PPARG*, *RET*, *PTEN* and *KRAS* mutations and 4 (12.9%) cases with *NRAS* mutations. One (3.22%) Bethesda III case had mutation in three genes; *NRAS* and the high risk mutations, *P13CA* and *TP53*. There were 4 (9.52%) classified as Bethesda IV; 2 (50%) had no mutations and 2 (50%) could not be analyzed. Of the 11 patients with mutations; 4 had surgery, 3 had no surgery and 4 had no follow-up. There was 2 cases of Hurthle cell carcinoma; 1 with *PTEN* mutation and 1 with mutation in *NRAS*, *P13CA* and *TP53* genes. There were 2 cases of PTC, 1 with *NRAS* mutation and 1 with *RET-PTC1* rearrangement. Of the 28 total cases without mutations; 4 had surgery, 21 had clinical and/or ultrasound follow-up and no thyroid surgery and 3 had no follow-up. Of the 4 cases without mutations which underwent surgery; 2 were Hurthle cell adenomas and 2 were oncocytic adenomatoid nodules, 1 in nodular goiter with incidental PTC (0.3 cm) in the contralateral lobe.

Conclusions: All 4 excised HCN cases with high risk mutations had a malignant outcome and all 4 excised HCNs with no mutations had a benign outcome. Two HCNs

classified as Bethesda II had *TSHR* mutation indicative of autonomously functioning thyroid nodules. ThyroSeq v1 NGS can aid in the pre-operative distinction of benign from malignant HCNs.

427 Cost-Effective Triaging of Ultrasound-Guided Fine Needle Aspiration (FNA) of Thyroid Nodules for Rapid On-Site Evaluation (ROSE)

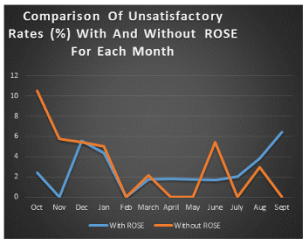
Renu Virk, Stefan Pambuccian, Swati Mehrotra, Lu Wang, Guliz Barkan. Loyola University Medical Center, Maywood, IL.

Background: There has been a steady increase in the number of thyroid FNA in recent years. ROSE is known to improve adequacy rates of ultrasound-guided (USG-guided) FNAs. Recent years have also witnessed a steady decline in compensation and relative value unit (RVU) of ROSE making it unaffordable for pathology practices to provide this service for all thyroid FNAs. Our institution adopted a cost-effective approach and opted to provide ROSE only for thyroid nodules meeting the following criteria: 1) Nodules with radiologic features suspicious for neoplasm/malignancy e.g. presence of microcalcifications 2) Prior unsatisfactory or atypical cytology results 3) Cervical lymph nodes for metastatic disease or suspected recurrent disease. Radiology department staff was adequately trained for specimen handling and smears preparation for cases not requiring ROSE.

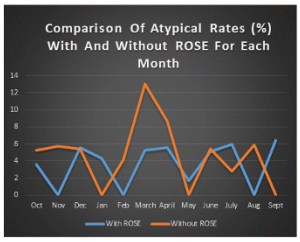
Design: This retrospective study was performed to compare the outcome of both groups with and without ROSE during an 11-month period from October 2013- September 2014. Outcome measurement included adequacy rates, unsatisfactory and atypia rates.

Results: A total of 991 USG-guided thyroid FNA were performed. ROSE was provided in approximately two third cases (n=620); the rest (n=371) were performed without ROSE. Comparable adequacy rates of 97% and 96% were achieved with and without ROSE respectively. Unsatisfactory rate for thyroid FNA with ROSE was 2.6% (n=16) compared to 3.5% (n=13) without ROSE (graph 1) whereas atypical rates (AUS and FLUS) with ROSE were 3.6% (n=22) compared to 5.4% (n=20) without ROSE (graph 2). The graphs demonstrate the trend and compares unsatisfactory and atypical rates between two groups for each month respectively. There is no statistical difference between two groups for unsatisfactory and atypia rates (p value: 0.4 and 0.19 respectively).

Graph 1



Graph 2



Conclusions: Comparable and similar adequacy, unsatisfactory and atypia rates can be achieved with and without ROSE on USG-guided thyroid nodules FNA after their appropriate triaging. This approach limits the number of cases requiring ROSE and allows better and cost-effective utilization of resources.

428 Cytopathologists' Use of the Indeterminate Diagnostic Categories "Atypical" (ATY) and "Suspicious for Malignancy" (SUSP) in the Cytologic Diagnosis of Solid Pancreatic Lesions

Renu Virk, Swati Mehrotra, Roberto Gamez, Guliz Barkan, Eva Wojcik, Stefan Pambuccian. Loyola University Medical Center, Maywood, IL.

Background: ATY and SUSP (categories III and V of the recent Papanicolaou Society of Cytopathology Guidelines for a Standardized Terminology and Nomenclature for Pancreatobiliary Cytology) are relatively vaguely defined categories used in pancreatic fine needle aspirates (FNAs) of solid lesions to diagnose cases showing cytologic features that are, in the cytopathologist's judgement worrisome, do not fit into benign/reactive changes, but are insufficient to allow a definite diagnosis of neoplasm/malignancy. The aim of this study was to determine the rate and variability of use of these diagnoses by cytopathologists and their potential correlates.

Design: All diagnoses made on pancreatic EUS-FNAs from 1/1/2001 to 9/15/2014 were tabulated by cytopathologists (CyP) and their rates of "unsatisfactory" (UNS), "negative for malignancy" (NEG), ATY, SUSP, and "positive for malignancy" were calculated,and rate of follow-up malignant diagnoses for ATY and SUSP. Spearman correlation was performed between the rates and with years of experience, yearly case load of pancreatic FNAs, and total pancreatic FNAs. The impact of cytology consensus review conference (held in 2009) on ATY and SUSP rates was calculated comparing the period before (2001-2009) to the period after the institution of this conference (2010-2014).

Results: Of the total 1622 pancreatic FNAs, 1121 (91.5%) cases diagnosed by 6 board-certified cytopathologists, results for which are shown in table 1.

Cytopathologists	Total Pancreas FNAs	Total solid lesions	UNSAT [%]	NEG [%]	ATY [%]	SUSP [%]	ATY+SUSP [%]	POS [%]	MALIGNANCY IN ATY+SUSP [%]
CyP1	281	204	13.7	32.3	5.9	0.9	6.9	45.5	55.0
CyP2	234	166	18.7	22.9	8.4	4.2	12.7	44.5	48.2
CyP3	115	80	13.8	23.8	8.8	3.8	12.5	50.0	70.0
CyP4	207	150	8.00	39.3	5.3	1.3	6.7	46.0	60.0
CyP5	402	319	12.5	38.2	7.2	2.5	9.8	38.9	55.0
CyP6	246	202	17.3	35.1	8.9	3.4	12.3	35.1	70.0
Lab average	1485	1121	14.0	32.9	7.4	2.4	9.9	42.4	60.0
2001-09	870	692	11.1	35.5	8.7	2.8	11.4	41.4	66.0
2010-14	758	539	17.6	29.3	5.8	2.0	7.8	44.3	61.0

The individual cytopathologists' ATY+SUSP rate did not correlate with %POS, years of experience or number of pancreatic FNAs diagnosed, but was inversely proportional to %NEG (r=-0.829, p=0.042) and directly proportional to %UNSAT (r=0.886, p=0.019).

Conclusions: Our experience shows that there is significant variation amongst pathologists for the diagnosis of ATY and SUSP; however, consensus review decreased the rate of such diagnoses. It also suggests that indeterminate diagnoses (ATY+SUSP) in FNAs of pancreatic solid lesions are made at the expense of NEG rather than POS or UNSAT diagnoses.

429 Molecular Testing of Different Cytologic Preparations – Which Yields the Best Results?

Marina Vivero, Vickie Y Jo, Monica Hollowell, Cibas Edmund, Neal Lindeman, Alarice Lowe. Brigham & Women's Hospital, Boston, MA; Boston Children's Hospital, Boston, MA.

Background: Molecular testing of lung adenocarcinoma specimens obtained via minimally invasive methods is increasingly important, especially in patients with advanced disease. Although studies have shown that cytologic specimens are amenable to molecular analysis, a systematic comparison of different cytologic preparations has not been performed. This study aims to compare the yield of positive molecular results among different cytologic preparations in patients with lung adenocarcinoma, with an additional focus on relative tumor cellularity and total tumor content.

Design: Archival cytologic slides (stained smears, liquid-based preparations, and cell block sections) were retrieved from patients with lung adenocarcinoma with a known EGFR, KRAS, or BRAF mutation. The proportion of tumor cells relative to total nucleated cells was quantified as <25%, 25-50%, or >50%, and total tumor cell content was scored as low or high (≤ or > 100 tumor cells, respectively) for each slide. The slides were tested for EGFR, KRAS, or BRAF mutations by direct sequencing and recorded as concordant or discordant with the known positive clinical result. Categorical analysis was performed using a 2-tailed Fisher exact test.

Results: 19 men and 32 women (mean age 64.5 years, range 35-95) contributed 54 specimens (3 broncho-alveolar lavages, 25 effusions, and 26 FNAs), yielding 91 preparations: 43 cell blocks (CB), 21 ThinPrep® slides (TP), 15 air-dried Hemacolor-stained smears (AD), and 12 alcohol-fixed Papanicolaou-stained smears (PAP). 89 preparations (98%) yielded informative molecular results. Overall, 71 (80%) study cases were concordant and 18 (20%) were discordant with clinical testing. When stratified by preparation method, there was no significant difference in the concordance rate for CB's (35/43, 81%), TP's (14/19, 74%), AD's (11/15, 73%), and PAP's (11/12, 92%,), p=0.5722. Concordance rates were also not significantly different between preparations with <25%, 25-50%, and >50% tumor cell proportion (5/8, 63% vs. 32/41, 78% vs. 34/40, 85%; p=0.2836). Concordance rates were significantly lower in preparations with fewer than 100 tumor cells (10/20, 50% vs. 54/61, 89%; p=0.0007).

Conclusions: CB, AD, PAP, and TP preparations are all valid substrates for molecular testing and yield results comparable to clinically-tested specimens. Tumor cell content affects testing results and should be recorded prior to molecular analysis. Slides with fewer than 100 tumor cells yielded fewer concordant results when compared to clinical specimens.

430 The Diagnostic Implications of GNAS Point Mutation in Pancreaticobiliary Neoplasia in FNA and Brush Cytology Specimens

Christopher Vytlacil, Jan Silverman, Sydney Finkelstein. Allegheny General Hospital, Pittsburgh, PA; RedPath Integrated Pathology, Pittsburgh, PA.

Background: Mutation detection of free DNA in cytocentrifugation supernatant fluid (CCSF) has been shown to complement cytology and improved the diagnosis of pancreaticobiliary cancer in FNA and brushing specimens. Optimal sensitivity for cancer detection depends on the correlation of mutational marker status with malignant transformation. GNAS oncogene point mutation has been recently described in association with intraductal papillary mucinous neoplasia (IPMN) which can involve both the pancreas and bile duct, potentially leading to adenocarcinoma. We present our experience with GNAS mutational analysis of CCSF to complement pancreaticobiliary cytology evaluation.

Design: Two pancreatic mass FNA/bile duct stricture brushing CCSF cohorts were studied: 1) 21 specimens with adenocarcinoma cytology, and 2) 123 specimens with high grade (HG; n=10) cytologic atypia, low grade atypia, benign, or insufficient cytology. Cell-free CCSF DNA was extracted and analyzed by PCR/capillary electrophoresis for KRAS/GNAS point mutation and loss of heterozygosity (LOH) with markers targeting 3p, 5q, 9p, 10q, 17p, 17q, 21q, 22q. KRAS, GNAS, and LOH mutational profiling was evaluated for its ability to detect cancer as an ancillary tool for cytology.

Results: Combined use of KRAS and LOH mutations detected all CCSF cohort 1 cytologically confirmed malignant samples as well as all 10 CCFS cohort 2 cases with high grade cytology (1 bile duct brushing, 9 pancreatic solid mass FNA). KRAS and

LOH showed equivalent cancer detection sensitivity (23/31, 74%) used alone. In 113 CCSF cohort 2 specimens lacking high grade cytology, KRAS & LOH markers were positive in 8 cases. 9p LOH proved to be the single most effective LOH marker. Of note, GNAS point mutation was not detected in any pancreatic solid mass FNA or biliary stricture brushing cytology specimens.

Conclusions: Molecular analysis of CCSF can detect mutations even in paucicellular and non-definitive pancreaticobiliary needle aspiration/duct brushing cytology specimens. Despite its established role in IPMN formation, GNAS was distinctly absent from malignant disease in contrast to that of KRAS and LOH mutations. Therefore, only finding a GNAS mutation in pancreaticobiliary needle aspiration/duct brushing cytology specimens can be helpful in diagnosing IPMN and in excluding malignant transformation.

431 Follicular Variant of Papillary Thyroid Carcinoma (FVPTC) Fine Needle Aspiration (FNA) Cytology Diagnoses Before and After the Implementation of the Bethesda System for Reporting Thyroid Cytopathology (BSRTC)

Lu Wang, Guliz Barkan, Swati Mehrotra, Renu Virk, Eva Wojcik, Stefan Pambuccian. Loyola University Medical Center, Maywood, IL.

Background: Papillary thyroid carcinoma (PTC) is the most commonly diagnosed malignancy in thyroid FNA. While the sensitivity of FNA in the diagnosis of the conventional PTC is high, its sensitivity in diagnosing FVPTC is much lower. The aspirates from FVPTC usually show less nuclear abnormalities than conventional PTC and frequently display a microfollicular pattern, resulting in an "indeterminate" rather than "positive" diagnosis. The aim of this study was to determine the impact of the BSRTC on the diagnoses made on FNAs preceding a histopathologic diagnosis of FVPTC.

Design: All cases diagnosed as FVPTC on surgical pathology material from 01/01/2004 to 7/15/2014 were identified through a search of our institution's electronic records; diagnoses made on thyroid FNAs performed up to 24 months previously were retrieved. Diagnoses made before and after the implementation of BSRTC at our institution (1/1/2011) were compared using the two-tailed Fisher exact test.

Results: 186 cases of FVPTC were diagnosed during the study period; of these, 136 (104 women and 32 men, aged 16-87) had an FNA performed within 24 months prior to surgery (mean 2.4 months); 73 were diagnosed before and 63 after the implementation of BSRTC. There were no significant differences between the ages (mean 49.88±14.28 vs. 49.76±14.28) or percentage of female patients (74% vs. 79%) between the two groups. After excluding unsatisfactory FNA specimens (4 pre- BSRTC, 1 post- BSRTC), the rates of diagnoses were:

	Benign	Atypical	Follicular Neoplasm	Suspicious for PTC	Positive for PTC
Before BSRTC	24.64%	1.45%	15.94%	18.84%	39.13%
After BSRTC	24.19%	20.97%	11.29%	9.68%	33.87%

The rate of cases diagnosed "atypical" in the pre-BSRTC terminology and "atypia of undetermined significance" or "follicular lesion of undetermined significance" (AUS/FLUS) in the post-BSRTC period differed significantly (p=0.0003). This increase was made at the cost of diagnoses of susp/pos PTC, which dropped from 58% to 43.5% (not significant, p=0.11).

Conclusions: Our study confirms the difficulties encountered in the diagnosis of FVPTC, since only 51% of all cases were diagnosed as either suspicious or positive for papillary carcinoma. The implementation of the BSRTC increased the diagnostic equivocation - a shift of the non-positive diagnoses towards "atypia" diagnoses.

432 Molecular Testing for EGFR Mutations and ALK Rearrangements in the Cytological Specimens

Xiaoyan Wang, Kristin Post, Shaoxiong Chen, Harvey Cramer, Robert Emerson, Howard Wu, Meehan Kari, Liang Cheng. Indiana University School of Medicine, Indianapolis, IN.

Background: Identifying for epidermal growth factor receptor (EGFR) mutations and translocations involving anaplastic lymphoma kinase (ALK) in lung cancers has become increasingly important because of successful targeted therapies. Cytology remains the initial approach in establishing the diagnosis of lung cancers due to the minimally invasive nature of procedure. This study is undertaken to investigate EGFR mutations and ALK gene rearrangements using cytological specimens from the patients with a diagnosis of primary or metastatic lung non-small cell carcinoma.

Design: Patients with primary or metastatic non-small cell lung carcinoma tested for EGFR mutations and/or ALK gene rearrangements were retrospectively identified from October 2011 to March 14 2014. A total 253 cases were submitted for EGFR mutational analysis and 214 cases for ALK analysis. The cytological specimen sources included lung, lymph node, liver, bone, adrenal gland, mesentery mass, and body fluid/effusions. EGFR mutations in the exons 18 -21 were analyzed (Qiagen, Valencia, CA). Fluorescence in situ hybridization (FISH) studies for ALK rearrangement inv(2)(p21;p23) were performed on the paraffin embedded cell block sections utilizing dual-color Vysis LSI ALK Break Apart Probe Kit (Vysis, Downers Grove, IL).

Results: Among 253 fine needle aspirate cases for EGFR analysis, 252 cases (246 from cell blocks, 6 from direct smears) had sufficient material for EGFR test. One case failed due to inadequate cellularity. Fourteen of 251 (6%) cases were positive for EGFR mutations, which included 6 cases of exon 19 deletions (del E746-A750), 7 cases of exon 21 (L858R) substitution mutations, and 1 case exon 21 (L861Q) substitution mutation. A total of 214 cases submitted for ALK analysis included 191 cases of fine needle aspirate, 20 cases of pleural fluid, 2 cases of pericardial fluid, and 1 case of bronchial washings. Six cases failed because of low cellularity whereas 208 of 214 cases had sufficient material for ALK FISH study. Seven of 208 cases (3%) revealed ALK rearrangement by FISH.

Conclusions: The current study demonstrates that cytological specimens can yield sufficient material for EGFR mutations and ALK rearrangement test. Our study reveals that 6% of EGFR mutation rate and 3% of ALK rearrangement rate in the cytology specimens from the patients with primary or metastatic lung non-small cell carcinoma.

433 Limitations and Pitfalls of Endoscopic Ultrasound-Guided Fine Needle Aspiration in the Diagnosis of Gastrointestinal Stromal Tumor

Natalie Webster, Mojtaba Olyaee, Ossama Tawfik, Fang Fan. University of Kansas Medical Center, Kansas City, KS.

Background: Gastrointestinal stromal tumors (GISTs) account for the majority of mesenchymal tumors in the GI tract. It is important to distinguish GIST from other spindle cell lesions because all GISTs carry some degree of malignant potential. EUS in combination with EUS-guided fine-needle aspiration (EUS-FNA) increases diagnostic accuracy, but results are variable. The goal of this study is to review our experience in the diagnosis of GISTs by EUS-FNA.

Design: EUS-FNAs of GI tract submucosal spindle cell lesions were identified from our cytology files between 2002 and 2014. The cases with a diagnosis of spindle cell lesion not otherwise specified (NOS), leiomyoma, or GIST were retrieved as well as age, gender, and location of the lesion. The surgical pathology follow-up of all cases was also reviewed if available.

Results: A total of 73 cases were retrieved from our cytopathology files, including 45 spindle cell lesions NOS (62%), 24 GISTs (33%), and 4 leiomyomas (5%). The patients included 27 males (37%) and 46 females (63%) with a mean age of 61 years (range 30-87). The most common location was gastric with 45 cases (62%) followed by esophageal with 14 cases (19%). 18 cases in the spindle cell lesion NOS group had surgical pathology follow-up which revealed 11 GISTs (5 low-risk, 2 intermediate risk and 4 high-risk), 5 leiomyomas, 1 submucosal lipoma and 1 metastatic renal cell carcinoma. Retrospective review of the cytology on the submucosal lipoma and the metastatic renal cell carcinoma cases identified scant spindle cell bundles most likely represent normal muscularis propria. One case in the leiomyoma group and 12 cases in the GIST group had surgical pathology follow-up which showed 1 leiomyoma and 12 GISTs (6 low-risk, 4 intermediate risk and 2 high-risk) respectively.

Conclusions: EUS-FNA cytology is accurate in diagnosing GI tract spindle cell neoplasms and the diagnosis of GIST is accurate when it is rendered. However, many GI tract spindle cell lesions are not further classified in our study due to lack of material for immunohistochemical stains on the cell block sections. There are no reliable diagnostic morphologic criteria for GIST. Therefore, an effort should be made during the procedure to request additional passes and to limit the amount of material for smears to increase the yield of cellblock preparation. Prominent normal muscularis propria poses an uncommon but potential diagnostic pitfall.

434 Diff-Quik® Stained Cytology Smear Slides as Primary or Supplemental Source for Molecular Analysis

Shuanzeng Wei, Zubair Baloch, Caren Gentile, Monica Shah, Matthew Hiemenz, Christopher Watt. Hospital of the University of Pennsylvania, Philadelphia, PA.

Background: In the current era of personalized medicine it is not unusual for molecular analysis to be performed on cytology specimens. The most commonly employed cytology preparation for these tests is cellblock; however, in a good proportion of cases this cannot be accomplished due to limited number of adequate lesional cells. In this study, we investigated if a single Diff-Qick stained slide prepared for assessing the adequacy / delivering preliminary diagnosis of fine-needle aspiration specimen (FNA) can serve as source for specific molecular test(s).

Design: The case cohort included 13 cases of melanoma and 7 cases of thyroid nodule FNA (6 papillary thyroid carcinomas & one hyperplastic nodule case). All diagnoses were confirmed by surgical pathology follow-up. All cases had more than one Diff-Quik stained slides and an accompanying FNA aspirate available for molecular analysis. The selected Diff-Quik® stained slide was reviewed to ensure that the specimen contained at least 10% lesional cells and greater than 200 tumor cells. The slide was digitally scanned at 400X for permanent electronic storage. After being decoverslipped in xylene, the cells were then scraped from the slide to produce a cell pellet; this was washed with 100% ethanol and digested with proteinase K in lysis buffer. The mixture was then applied to a Qiagen MinElute spin column to purify the DNA. The DNA was washed, and then eluted from the column using the QIAamp DSP FFPE Tissue Kit. All cases were pyrosequenced to evaluate for the presence of BRAF codon 600 mutations including V600E (c.1799T>A), V600K (c.1798_1799GT>AA) and V600R (c.1798_1799GT>AG). For NRAS mutations in codons 12 and 13 in exon 2 and codon 61 in exon 3, thyroid FNA specimen were evaluated by pyrosequencing.

Results: Adequate quantity and quality of DNA was achieved from both FNA aspirate and Diff-Quik slide specimen. The BRAF mutation was demonstrated in 8 of 13 cases of melanoma and 2 of 7 cases of thyroid FNA using Diff-Quik® slide specimen; these were confirmed by using FNA aspirate. No NRAS mutations were identified in the thyroid FNA specimens.

Conclusions: Adequate quantity and quality of DNA can be obtained from Diff-Quik® cytology smear slides. With appropriate digital archiving, Diff-Quik® slides can serve as a reliable primary or supplemental source for molecular tests in cases with limited cellularity cellblock specimens.

435 Whole Slide Image With Image Analysis of Atypical Bile Duct Brushing: Quantitative Features Predictive of Malignancy

R Cody Weimholt, Brian Collins. Washington University, St. Louis, MO.

Background: Whole slide images (WSI) involve digitally capturing microscopic glass based preparations for computer based viewing. WSI are amenable to quantitative image analysis of the cellular morphologic features. Bile duct (BD) brushing can show morphologic features which are categorized as indeterminate for malignancy. This study aims to evaluate quantitative morphologic features of atypical categories of BD brushing by WSI image analysis for the identification of criteria predictive of malignancy.

Design: Over a 3 year period, the pathology database was searched for BD brush specimens with indeterminate diagnostic categorization (atypical to suspicious) and corresponding tissue biopsy diagnostic for malignancy. BD brush slides were captured as WSI (Leica) and then subjected to image analysis (ImageScope). Ten well visualized groups with morphologic atypical features were selected per case and had quantitative analysis performed for group area, individual nuclear area, number of nuclei per group, N:C ratio and nuclear size differential.

Results: There were twenty eight cases identified which each had a corresponding confirmatory surgical pathology diagnosis. 17 were categorized as atypical and 11 as suspicious. The total nuclei per group average ranged from 5.2 to 29 per case and the nuclear area average ranged from 35.7 to 109.7 µm2 per case. The nuclear size differential ranged from 36.3 to 110.7 µm2 per case and the N:C ratio average ranged from 0.39 to 0.66 per case. Cases were aggregated into cytologic diagnostic categories of atypical and suspicious. The average nuclear area was 63.7 µm2 for atypical and was 80.1 µm2 for the suspicious (+difference 16.4 µm2; p=0.002). The nuclear size differential was 69.7 µm2 for the atypical and 88.4 µm2 for the suspicious (+difference 18.8 µm2; p=0.009). The average total nuclei per group showed only a 0.7 difference and the average N:C ratio showed only a 0.01 difference. An average nuclear area greater than 70 µm2 had a 3.2 risk ratio for suspicious categorization.

Conclusions: The quantitative criteria findings as measured by image analysis on WSI showed that cases categorized as suspicious had more nuclear size pleomorphism (+18.8 µm2) and larger nuclei (+16.4 µm2) than those categorized as atypical. WSI and morphologic image analysis can demonstrate quatitative statistically significant differences between atypical and suspicious BD brushings and provide objective criteria which support the diagnosis of carcinoma.

436 Human Papillomavirus Infection, BK Virus Infection and Cervical Intraepithelial Lesions in Female Renal Transplant Recipients: An Institutional Review

Baoying Weng, Mayes Dormosh, Anne King, Adele Kraft, Celeste Powers. Virginia Commonwealth University Health Science, Richmond, VA.

Background: Immunocompromised patients such as renal transplant recipients have an increased incidence of cervical neoplasms. Association between high risk human papillomavirus (HR-HPV) infection and cervical intraepithelial neoplasia (CIN) has been well established. Renal transplant recipients receiving immunosuppressive therapy are prone to viral infection. Recent research shows that BK virus (BKV) can co-exist with HR-HPV in cervical specimens. The objective of this study is to investigate the incidence of HR-HPV and BKV infection and cervical intra-epithelial lesions in female renal transplant patients.

Design: Using the transplant center database we identified all women that underwent renal transplant at our institution and had Pap test performed between 2009 and 2014. Colposcopy and biopsy were performed on patients positive for cervical intraepithelial lesion on Pap test. HR-HPV was detected in cervical cytology specimens with Hybrid Capture II and BK virus infections were analyzed in the blood with real time PCR.

Results: From a total of 298 female renal transplant patients 33 had Pap test performed after transplantation. One patient had high grade intraepithelial lesion, 10 patients had low grade intraepithelial lesion and 22 patients were negative for intraepithelial lesion or malignancy. The rate of cytological abnormalities (11/33, 33%) was significantly higher than in the general population (4.5 -8.5%). Colposcopy revealed 4 CIN in 8 patients with abnormal cytology. HR-HPV was detected in 7 of 20 (33%) tested patients and in 6 of 8 (75%) patients with abnormal cytology. BKV was detected in 14 of 33 (42%) tested patients and in 4 of 11 (36%) patients with abnormal cytology. BKV was present in 4 of 7 (57%) HR-HPV positive patients and in 7 of 13 (54%) HR-HPV negative patients. One patient with abnormal cytology was BKV positive and HR-HPV negative.

Conclusions: Preliminary data suggests that female renal transplant patients undergoing immunosuppressive therapy are at increased risk of developing CIN. BKV can present in HR-HPV infected patients. All the patients using immunosuppressive agents should be followed-up closely with Pap test and colposcopy evaluation. Further evaluation of the clinical significance of HR-HPV and BKV co-infection in female renal transplant patients is needed as BKV may be a potential co-factor for HR-HPV infection in the development of cervical lesions.

437 Use of BRAF Immunohistochemistry on Cytologic Direct Smears of Papillary Thyroid Carcinoma

Sara Wobker, Lawrence Kim, Trevor Hackman, Leslie Dodd. University of North Carolina, Chapel Hill, NC.

Background: Papillary thyroid carcinoma (PTC) is the most common subtype of thyroid carcinoma. Mutations of BRAF are found in up to 46% of these tumors. There is conflicting evidence about the prognostic value of BRAF mutations in PTC, with some retrospective studies showing higher mortality in BRAF-mutant cases. Cytologic smears may provide adequate material for the molecular analysis of BRAF mutations in PTC by immunohistochemistry. The specific mutation is a nucleotide transversion resulting in a valine to glutamate (V600E) substitution. An immunohistochemical stain for BRAF is commercially available for the V600E mutation and validated in surgical specimens. Our study will assess the performance of BRAF IHC on cytologic direct smears.

Design: FNA specimens with paired surgical resection specimens with a diagnosis of PTC were identified. For inclusion, the case must have sufficient cellularity on a Pap stained cytologic smear to permit staining with BRAF IHC. We first performed immunostaining on the surgical specimens to determine mutation status. We then stained the corresponding cytologic smears for BRAF to assess correlation. Positive cytologic controls were melanomas confirmed to have V600E mutation by Sanger sequencing.

Results: We identified 46 paired cytology and surgical specimens. Of these, 22 surgical specimens were positive for BRAF (47.8%). The paired cytologic smears of these cases revealed 65.3% concordance with 30 of 46 staining similarly for BRAF. Using the surgical cases as a gold standard, the sensitivity of BRAF staining on cytologic smears was 63.6% and the specificity was 58.3%. Concordance rates were highest in FNA specimens diagnosed as Positive for malignancy or Suspicious for malignancy (75% and 85.7%, respectively) and lowest for AUS (40%).

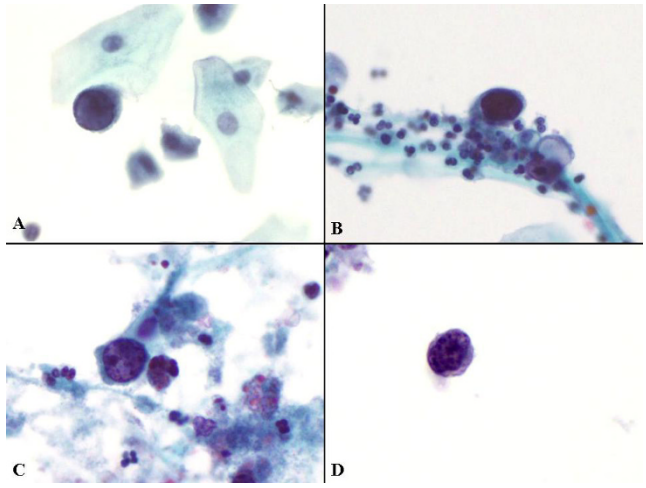
Conclusions: We were able to use cytologic smears to detect BRAF mutations in our study sample with 65.3% concordance. Limitations to the interpretation of smears include low cellularity and obscuring blood clot or colloid. Cases that were Suspicious or Positive for malignancy on FNA were most likely to be BRAF concordant. This association may serve as a proxy for adequate cellularity. Our study shows proof of concept and will require more investigation for optimization. With further refinement, it is possible that BRAF immunohistochemistry can be applied prospectively to thyroid FNAs for risk stratification.

438 Urine Cytology: Does the Number of Atypical Urothelial Cells Matter for Distinguishing the "High-Grade Urothelial Carcinoma (HGUCA)" From the "Suspicious for HGUCA" Cytological Categories?

Bin Xu, Manon Auger, Fadi Brimo. McGill University Health Center, Montreal, QC, Canada.

Background: This study aims to evaluate whether the number of atypical urothelial cells is an important criterion in separating the category of 'positive for HGUCA' from what is referred to as 'suspicious for HGUCA' or 'atypical urothelial cells, cannot exclude HGUCA/AUC-H'.

Design: 103 consecutive urine cytology specimens with 'atypical', 'suspicious'or 'positive' original diagnoses that had follow-up biopsies were included; they were fully reviewed and the number of atypical cells was recorded. Atypical cells were defined as intermediate cells with hyperchromatic nuclei and an N/C ratio> 0.7. Four major types of atypical cells were identified (A-D)



Results: In more than 90% of cases more than one atypical cell type was present. Overall, follow-up biopsy was benign in 11 (11%), low grade UCA in 20 (19%) and HGUCA in 72 (70%) of cases. Cases with ≤ 10 atypical cells (n=38) had significantly lower rates of subsequent HGUCA than those with >10 atypical cells (58% versus 77%. p=0.04). In comparison, the association with any subsequent UCA (low- and high- grade) was not significantly different (84% versus 92%. p=0.2). Cases with ≤ 5 atypical cells (n=26) showed similar prediction rates for HGUCA than those with 6-10 atypical cells. Among cases with ≤ 10 atypical cells in which only one atypical cell type was present, the incidence of subsequent HGUCA was highest for atypical cell type D (5/7 cases) in comparison to the other cell types (0/1 for type A and 1/4 for type C).

Conclusions: The number of atypical urothelial cells is an important criterion to classify urine cytology specimens into the 'positive' or the 'suspicious'/AUC-H categories. A cut-off number of 10 cells to emit a definitive diagnosis of HGUCA seems valid from the clinical standpoint .

Figure 1. All cells have N/C ratio>0.7 and hyperchromatic nuclei. Absence of chromatin details (type A, B) with (type B) or without (type A) irregular nuclear membranes. Presence of coarse irregular chromatin (types C, D) with (type D) or without (type C) irregular nuclear membranes.

439 Clinical, Cytologic and Immunohistochemical Features of Soft Tissue Perineurioma: A Comparative Analysis With Benign and Malignant Mimics

Eric Yang, Jason Hornick, Xiaohua Qian. Brigham & Women's Hospital, Boston, MA.

Background: Soft tissue perineurioma (STP) is an uncommon benign soft tissue tumor derived from perineurial cells of the peripheral nerve sheath. The perineurial nature of this tumor type is supported by its nearly invariable expression of EMA. Very few FNA specimens of STP have been described and their cytomorphologic features remain incompletely characterized. Furthermore, the distinction of STP from its benign (cellular myxoma; CM) and malignant (low-grade fibromyxoid sarcoma; LGFMS) mimics can be challenging. Therefore, the purpose of this study was to compare the clinical, cytologic, and immunohistochemical features of STP, CM, and LGFMS, and to propose a practical and clinically meaningful diagnostic approach to these low-grade myxoid spindle cell neoplasms in biopsy material.

Design: All cases of STP (5), CM (15) and LGFMS (4) from 2005-14 with FNA/biopsy and resections were included. Slides were reviewed by 3 pathologists, and discordances were resolved by consensus review. Chi-square test was used to determine statistical significance.

Results:

Table 1: Comparison between soft tissue perineurioma and cytologic mimics.

		Soft Tissue Perineurioma (n=5)	Cellular Myxoma (n=15*)	Low-grade Fibromyxoid Sarcoma (n=4)
Clinical	Median Age	45	56	48
	[range]	[42-60]	[26-77]	[21-68]
	Sex	3F:2M	11F:4M	2F:2M
	Site	Thigh (3), toe (1), perirectal soft tissue (1)	Thigh (10), buttock (2), calf (1), arm (1), chest wall (1)	Calf (1), arm (1), buttock (1), groin (1)
	Mean Size	6.6 cm	6.5 cm	6.4 cm
Cytologic Features	Myxoid matrix	1 (20%)	11 (73%)**	1 (25%)
	Cytologic atypia	0 (0%)	0 (0%)	2 (50%)
	Spindled nuclei	3 (60%)	8 (53%)	2 (50%)
	Bipolar Cytoplasmic processes	1 (20%)	0 (0%)	0 (0%)
	Vessels	1 (20%)	5 (33%)	1 (25%)
IHC	EMA(+)	5 (100%)	8 (57%)	3/3 (100%)
	Claudin-1(+)	2 (40%)	4 (28%)	1/1 (100%)
	S100(+)	0 (0%)	0 (0%)	0/4 (0%)
	CD34(+)	4 (80%)	12 (85%)	0/3 (0%)
	MUC4(+)	0 (0%)	0 (0%)	3/3 (100%)

*14 CM available for IHC
**Statistically significant (p<0.05)

Cytomorphologic features of STP included clusters of slender cells with no atypia, bipolar cytoplasmic processes, and a limited amount of myxoid to collagenous matrix. STP could be distinguished from CMs by the relative lack of myxoid matrix (20% STP; 73% CM; $p<0.05$), but these tumors were otherwise remarkably similar. All STP and CM lacked cytologic atypia, whereas 50% of LGFMS showed mild atypia. EMA was positive in all STPs, but was also at least focally positive in 57% of CM and 100% of LGFMS (100% sensitivity, but 35% specificity). MUC4 was negative in all CM and STP but positive in all LGFMS. Interestingly, 1 case initially diagnosed as STP was MUC4 positive and was therefore reclassified as LGFMS.

Conclusions: When evaluating the FNA/biopsy specimen of a low-grade myxoid spindle cell neoplasm, the cytomorphology should be examined carefully with attention to the background matrix. Our findings argue against relying on EMA for the diagnosis of STP in biopsy material. We further recommend performing immunohistochemistry for MUC4 to exclude LGFMS. If MUC4 is negative, the most practical and clinically useful diagnosis is "*benign* myxoid spindle cell neoplasm".

440 Epithelial Cell Abnormalities in Pap Smears From HPV Vaccinated Women

Howard Yang, Sharon Nofech-Mozes, Valerie Dube, Nancy Durand, Michael Shier, Gita Desai, Zeina Ghorab. Sunnybrook Health Sciences Centre, University of Toronto, Toronto, ON, Canada.

Background: Human Papilloma Virus (HPV) is an important cause of cervical cancer. Since 2007, the HPV vaccination program has been publicly funded for Grade 8 girls and approved for females aged 9-45. Only limited data is available on the frequency of epithelial cell abnormality (ECA) detected on Pap smears in vaccinated women.

Design: In 2011 fields documenting the vaccination status were added to our Pap test requisition to allow for prospective data collection. We retrospectively searched all Pap smear reports between 1/2011 and 8/2014 for clinical information on the vaccination status. The frequency of ASCUS, LSIL, HSIL, glandular lesions and carcinoma was compared between vaccinated and non vaccinated women.

Results: HPV vaccination status was provided in 11,722 of 53,810 pap smears: 2,204 vaccinated, 9,518 not vaccinated. 279 specimens were unsatisfactory. Table 1 summarizes the frequency of ECA. Vaccinated women were younger ($p < 0.001$) had more LSIL ($p < 0.001$) and comparable HSIL ($p = 0.67$). Squamous cell carcinoma (SCC) occurred in a patient vaccinated 3 years prior to diagnosis of invasive cancer that was tested positive for a non16/18 HPV serotype.

Conclusions: HPV vaccination status was provided mainly by colposcopy clinics, therefore our results reflect the diagnoses in high-risk population that may have been exposed prior to vaccination and had high rates of significant epithelial abnormalities. These findings underscore the importance of continuous screening in vaccinated women.

Table 1. Population characteristics and distribution of cytological findings

	Vaccinated	Not vaccinated	Unknown
PopulationCharacteristics			
Mean Age - yr	30.5	39.6	38.5
Colposcopy Related Specimens (N)	1,924	5,789	11,417
General / GYN Specimens (N)	280	3,729	30,671
Cytological Findings (N) (%)			
Negative	1,309 (59.69%)	6,725 (71.17%)	33,542 (80.11%)
ASCUS	283 (12.90%)	859 (9.09%)	3,060 (7.31%)
LSIL	501 (22.85%)	1,399 (14.81%)	4,102 (9.80%)
HSIL	96 (4.38%)	433 (4.58%)	1,068 (2.55%)
AIS/AGC	1/2 (0.05%/0.09%)	4/18 (0.04%/0.19%)	7/52 (0.02%/0.12%)
Adenocarcinoma/SCC	0/1 (0%/0.05%)	1/7 (0.01%/0.06%)	8/13 (0.02%/0.03%)
Non-cervical malignancies	0 (0%)	3 (0.03%)	18 (0.04%)

ASCUS–atypical squamous cells of undetermined significance, LSIL–low-grade squamous intraepithelial lesion, HSIL–high-grade intraepithelial lesion, AIS–adenocarcinoma in-situ, AGC–atypical glandular cells, SCC–squamous cell carcinoma

441 The Utility of Gene Expression Classifier (Afirma, Veracyte, Inc.) Testing in Indeterminate Thyroid Nodules: A Follow-Up Study

Sung Eun Yang, Jianhua Zhang, Peggy Sullivan, Jianyu Rao, Rekha Govind, Mary Levin, Neda Moatamed. University of California, Los Angeles, CA.

Background: Thyroid fine-needle aspiration (FNA) has a pivotal role in the initial clinical evaluation of thyroid nodules. Up to 30% of cases are diagnosed as indeterminate by FNA (including atypia of undetermined significance (AUS), follicular lesion of undetermined significance (FLUS), suspicious for a follicular neoplasm (SFN), and follicular neoplasm (FN)). Two-thirds of these cases will have a post-operative benign diagnosis. The gene expression classifier (GEC) test (Afirma, Veracyte, Inc.) is a molecular test for thyroid FNA cases with indeterminate cytological diagnoses.

Design: Retrospective analysis of all thyroid FNAs from a single institution during a 19 month period was performed. Cases were included if the patient had an indeterminate cytological diagnosis (AUS, FLUS, SFN, or FN) with GEC results. 204 cases met these inclusion criteria (FLUS/AUS, n = 181; SFN/FN, n= 23). Correlation with surgical follow-up (SFU), when available, was performed.

Results: For the FLUS/AUS category, the GEC results were non-diagnostic in 7% (13/181), benign in 43% (77/181), and suspicious in 50% (91/181) of cases. For the SFN/FN category, the GEC results were non-diagnostic in 9% (2/23), benign in 39% (9/23) and suspicious in 52% (12/23) of cases.

Sixty cases had SFU available (GEC suspicious, n=57; GEC benign, n=3). Of the cases with a suspicious GEC result, 58% (33/57) had a malignant final diagnosis, and 42 % (24/57) had a benign final diagnosis. Of the cases with a benign GEC result, 100% (3/3) had a benign final diagnosis.

FLUS/AUS cases with a "suspicious" GEC result had 38% (18/47) benign SFU and 62% (29/47) malignant SFU (14 follicular variant of papillary thyroid carcinoma (FVPTC), 10 classic PTC, and 5 micropapillary thyroid carcinoma (MPTC)).

FN/SFN cases with a "suspicious" GEC result had 60% (6/10) benign SFU and 40% (4/10) malignant SFU (2 FVPTC, 1 microinvasive follicular carcinoma and 1 Hurthle cell carcinoma).

Conclusions: The GEC test has high sensitivity (100%), high negative predictive value (100%), low positive predictive value (58%), and low specificity (11%). The utility of GEC in cases diagnosed as FN/SFN appears limited, since even with suspicious GEC results, these lesions had benign surgical follow up in 60% of the cases.

On the other hand, FLUS/AUS cases with a suspicious GEC result had a malignant SFU in 62% of cases, the majority of which were FVPTC. In this area where cytology has some limitation, additional studies demonstrating the utility of GEC testing is warranted.

442 Prevalence and Genotype Distributions of HPV Infection in China: Analysis of 51345 HPV Tests From China's Largest CAP Certified Laboratory

Zhengyu Zeng, Huaitao Yang, Zaibo Li, Xuekui He, Xianmei Chen, Xiaolei Guo, Shangwei Wu, Chengquan Zhao. KingMed Center for Clinical Laboratory Co.,Ltd, GuangZhou, Guangdong, China; Cincinnati Medical Center, Cincinnati, OH; Ohio State University Wexner Medical Center, Columbus, OH; Magee-Womens Hospital of University of Pittsburgh Medical Center, Pittsburgh, PA.

Background: The prevalence of cervical HPV infection varies greatly worldwide. The data of HPV genotypes are limited in China.

Design: A retrospective database search from 2011-1 to 2014-6 was conducted in the Lab to analyze HPV testing results by Tellgen*plex*™ 26 HPV Genotyping Panel (TELLGEN, China), a multiple PCR method to detect 16 HR HPVs, 6 LR HPVs and 4 HPVs (26, 53, 55, 83) with undetermined risk.

Results: 51,345 women were tested by Tellgen*plex*™ 26 HPV Genotyping Panel. Samples were collected from 364 hospitals, clinics, and PE centers in Guangdong (55%) and other provinces (45%). The overall prevalence of HPV was 26.0% (20.7% for HR-HPVs, 1.5% for four undetermined types, and 7.8% for LR-HPVs). Multiple

HPV types infection accounts for 20% of HPV positive cases. HPV 52 and 16 were the most common HR HPV (5.1% and 4.8%), while HPV 18 was in the 6th position with only 1.6%. When women were divided into three age groups: <30, 30-49, ≥50 years, HR-HPV positivity was the highest in women <30 years, but lowest in women 30-49 years. The distribution of HR-HPV genotypes among these three age groups was different. The first six most common HR-HPV types in each group are as followings: 52, 16, 58, 59, 39, 56 in women <30 years; 52, 16, 58, 18, 39, 56, in women 30-49 years; 16, 52, 58, 56, 18, 68 in women ≥50 years.

Genotype	Positive cases	Prevalence(%)
16	2446	4.8
18	801	1.6
26*	29	0.1
31	454	0.9
33	631	1.2
35	213	0.4
39	858	1.7
45	198	0.4
51	613	1.2
52	2595	5.1
53*	338	0.7
55*	341	0.7
56	877	1.7
58	1504	2.9
59	779	1.5
66	410	0.8
68	719	1.4
73	16	0
82	385	0.7
83*	85	0.2
LR Type		
6	1732	3.4
11	1084	2.1
40	219	0.4
42	147	0.3
44	389	0.8
61	685	1.3

Conclusions: HPV type 52 is the most commonly identified HR-HPV, followed by HPV 16, 58, 56, 39, 18. The distribution of HR-HPV is different between China and Western countries. New prophylactic vaccines including HPV 52 and 58 may be necessary in China.

443 Prevalence of High Risk Human Papilloma Virus Infection in China: Analysis of 589,033 HPV Test Results From China's Largest CAP Certified Laboratory

Zhengyu Zeng, R Marshall Austin, Xuekui He, Xianmei Chen, Xiaolei Guo, Shangwei Wu, Chengquan Zhao. KingMed Center for Clinical Laboratory Co.,Ltd, GuangZhou, Guangdong, China; Magee-Womens Hospital of University of Pittsburgh Medical Center, Pittsburgh, PA.

Background: This study of HPV test results extends previously reported findings on cervical cytology performance in China's largest independent laboratory with ccreditation under the international LAP of the CAP.

Design: A retrospective database search from 2007-1 to 2014-6 was conducted to analyze hrHPV test results by either HC2 or Multiplex PCR fluorescence test (MPFT). HPV test samples were collected from 886 hospitals, clinics, and PE centers. More than 80% HPV tests were ordered by clinicians as primary cervical screening tests.

Results: During the study period, 573, 433 HC2 and 15, 600 MPFT HPV tests were performed. The average ages of the tested women were 35.7 years with HC2 and 37.2 years with MPFT. The overall HPV positive rate was 22% with HC2, significantly higher than the 17.4% with MPFT ($p<0.001$). HPV positive rates were significantly different in varying age groups ($p<0.01$), highest in women 10-19 years, second highest among women 60-69 years. The total volume of HPV tests per year steadily increased. The overall HPV-positive rate, however, decreased from 25.3% in 2007 to 20.1% in 2013, which may reflect increased screening of lower risk patients.

Age group	HC2			MPET		
	Case#	Positive#	%	Case#	Positive#	%
10-19	6649	2454	36.9	259	94	36.3
20-29	148442	36653	24.7	2914	635	21.8
30-39	196873	39404	20.0	5215	780	15.0
40-49	149613	30207	20.2	5427	818	15.1
50-59	30834	7202	23.4	804	176	21.9
60-69	5588	1645	29.4	120	39	32.5
≥70	1211	335	27.7	21	2	9.5
Unknown	34223	8075	23.6	840	163	19.4
Total	573433	125975	22.0	15600	2707	17.4

Conclusions: HC2 HPV detection rates exceeding 20% in all age groups of over 500,000 tested Chinese women were higher than that reported in the USA. PCR HPV detection rates were slightly lower than HC2 HPV detection rates in every age group. The high HPV infection rate in Chinese patients likely reflects limited cervical screening and treatment of precancer in China, correlated with the high rates of cervical cancer still prevailing through much of China. Many Chinese physicians already appear to be using HPV testing as a primary cervical screening test.

444 Significance of Atypical Endometrial Cells in Women Aged Younger Than 40 Years

Yaxia Zhang, Charles Biscotti, Hui Zhu, Fadi Abdul-Karim. Cleveland Clinic, Cleveland, OH.

Background: Benign appearing endometrial cells in Pap tests from women under the age of forty are not reported; however, atypical endometrial cells (AEM) are reported at any age. AEM have been associated with endometrial malignancy with reported rates ranging from 9-52%. However, most studies analyzed only patients older than 40 years. Endometrial carcinoma rarely affects young women. About 2-14% of endometrial carcinomas occur in women younger than 40 years, with a small subset of these tumors related to Lynch syndrome. This study investigates the significance of AEM in Pap test samples from women under 40 years of age, including an analysis of Lynch syndrome screening.

Design: Our laboratory's gynecologic cytology data base was searched for samples from 2000 to 2014 using the keywords "atypical endometrial cells" or "atypical glandular cells favor endometrial origin" in women younger than 40 years. Available ThinPrep slides were reviewed. The electronic medical record provided the clinical presentation, followup endometrial biopsy, treatment, and follow-up data. Endometrial carcinoma tissue sections were screened for Lynch syndrome using either immunohistochemistry (IHC) for mismatch repair protein expression or PCR-based microsatellite instability analysis.

Results: The search yielded63 cases. Patients' ages ranged from 17-39 years (mean 32.6). 11 patients had no endometrial biopsy followup. 9 (17.3%) of 52 patients with follow-up biopsies had premalignant (atypical hyperplasia = 5) or malignant lesions (endometrioid adenocarcinoma =4). 16 (30.8%) patients had other endometrial pathology including: hyperplasia without atypia (2), endometrial polyp (4), chronic endometritis (6), and disordered endometrium (4). 27 (51.9%) patients had proliferative or secretory endometrium. All 4 endometrioid adenocarcinomas had negative Lynch syndrome screening (microsatellite stable by PCR 1 case and normal by IHC 3 cases). The 9 patients with premalignant or malignant lesions (8 white, 1 black) were overweight or obese (BMI, 25-45, mean 39), and 6/9 presented with irregular menstrual cycles, abnormal bleeding. The patients with benign endometrial biopsy had BMI ranged from 19-50, with mean 30.

Conclusions: Our data support endometrial sampling in women younger than 40 years with AEM in Pap tests. Almost half (48.1%) of our patients, with follow up sampling, had endometrial pathology including 17.3% with malignant or premalignant lesions. These patients had a clinical profile characteristic of excess estrogen exposure and negative Lynch syndrome screening.

445 Comparison of Cervista and Hybrid Capture 2 HPV Detection Rates in Patients With LSIL and HSIL Cytology Results From the Same FDA-Approved Collection Vial

Chengquan Zhao, R Marshall Austin. University of Pittsburgh Medical Center, Pittsburgh, PA.

Background: New guidelines recommend women 30 to 65 with negative co-testing results should be rescreened in 5 years. Data remain very limited comparing HPV detection rates in SIL Paps when HPV testing is performed using different FDA-approved HPV tests.

Design: A retrospective search was conducted to identify women with LSIL and HSIL ThinPrep Pap and concurrent hrHPV test from 2010-7 to June 2014-6. Histopathologic follow-up results were obtained for the cases with HPV-negative HSIL Pap. HPV testing was performed by HC2 from 2010-7 to 2013-6 and by Cervista from 2013-7 to 2014-6.

Results: 1864 patients with LSIL and 216 with HSIL Pap and concurrent HC2 HPV test results were identified. HC2 HPV-positive rates were 81.9% and 94.4% in LSIL and HSIL cases, respectively. 1137 patients with LSIL and 116 patients with HSIL Pap with concurrent Cervista HPV test results were identified. Cervista HPV-positive rates were 77.3% and 90.5% in LSIL and HSIL cases, respectively. The HC2 HPV-positive LSIL rate was significantly higher than the Cervista HPV-positive LSIL rate. HPV positive rates were the lowest in women ≥50, 73.3% in LSIL and 87.2% in HSIL

respectively. Among 23 HPV-negative HSIL patients, 21 (11 Cervista-negative and 10 HC2-negative) had histopathologic results within one year: CIN2/3 in 28.6%, CIN1 in 47.6%, and negative in 23.8%.

LSIL							
	HC2			Cervista			Average HPV
	Case#	Positive#	%	Case#	Positive#	%	positive (%)
	335	301	89.9	265	230	86.8	88.5
	1175	958	81.5	635	484	76.2	79.7
	354	268	75.7	237	165	69.6	73.3
Total	1864	1527	81.9	1137	879	77.3	80.2
HSIL							
	HC2			Cervista			Average HPV
	Case#	Positive#	%	Case#	Positive#	%	positive (%)
	25	25	100	19	16	84.2	93.2
	157	149	94.9	84	78	92.9	94.2
	34	30	88.2	13	11	84.6	87.2
	216	204	94.4	116	105	90.5	93.1

Conclusions: HC2 HPV-positive LSIL and HSIL rates were slightly higher than Cervista HPV-positive LSIL and HSIL rates, with only the significant difference in HPV-positive LSIL rate. Additional studies on positive and negative predictive values, sensitivity, and specificity are needed to fully assess differences in HPV test performance. Data in this study does not support allegations that HPV-positive rates with the Cervista method may be too high (Am J Clin Pathol 2010; 134:193-199).

446 HSIL Misinterpretation Papanicolaou Test Rates in the College of American Pathologists PAP Education and PAP Proficiency Test Program in 2013

Chengquan Zhao, Barbara Crothers, Mohiedean Ghofrani, Mojtaba Hussain, Fang Fan, Idris Ocal, Diane Davey. University of Pittsburgh Medical Center, Pittsburgh, PA; Water Reed National Military Medical Center, Bethesda, MD; PeaceHealth Lab, Vancouver, WA; University of Central Florida, Orlando, FL; Unisersity of Kansas Medical Center, Kansas City, KS; Mayo Clinic, Scottsdale, AZ; University of Central Florida College of Medicine, Orlando, FL.

Background: Misinterpretation of HSIL is an important problem in daily practice and in proficiency testing (PT). This study is to investigate variables related to misinterpretions of HSIL.

Design: We analyzed the 2013 CAP PAP Proficiency Test (PAP PT) and PAP educational programs (PAP-Edu) for misinterpreted HSIL cases and related variables, such as Pap preparation types, test types, and personnel.

Results: There were 26,122 responses for HSIL slides, including 11,397 in PAP PT and 14,725 in PAP Edu. Overall, 2672 (10.2%) responses for HSIL were misclassified as no response (0.2%), unsatisfactory (0.3%), negative (2.6%),or LSIL (6.9%). More CP slides were misclassified (12.3%) than ThinPrep (8.5%), but less than SurePath (14.7%; P=0.004). Cytotechnologists were significantly more likely to misclassify HSIL (11.5%) than pathologists (9.1%; $P < 0.001$) Overall, the misclassification rate for HSIL was lower in PAP PT (3.9%) than in PAP Edu (15.1%, P <0.001). The most common interpretation for a HSIL reference diagnosis was LSIL;negative or unsatisfactory interpretations accounted for 0.9% and 5.0% in PAP PT and PAP Edu, respectively. 2.6% pathologists and 5.5% cytotechnologists misclassified HSIL in PAP PT (P<0.001) and 14.4% pathologists and 15.9% cytotechnologists misclassified HSIL in PAP Edu (P<0.01).

Categories	Pap PT (%)	Pap Edu (%)	Total
Unsat	0 (0)	70 (0.5)	70
Negative	103 (0.9)	658 (4.5)	761
LSIL	343 (3.0)	1455 (9.9)	1798
HSIL	10950 (96.1)	12500 (84.9)	23450
No response	1 (0)	42 (0.3)	43
Total	11397	14725	26122

Table

Conclusions: LSIL is the most common misclassified category for HSIL. The concordance of pathologists for a HSIL interpretation is better than for cytotechnologists and may relate to differences in reporting responsibilities and PT grading criteria. SurePath had the highest misintepretation rate of HSIL. The HSIL misinterpretation rate is higher in PAP Edu than PAP-PT for both pathologists and cytotechnologists. This may represent a defensive strategy by participants in the test-taking environment, or reflect differences in selection criteria for slide validation and inclusion into PAP PT versus PAP Edu.

447 Cobas HPV Test Performance for High-Grade Squamous Intraepithelial Lesion (HSIL): Analysis of 130,648 Pap Tests With 1,654 Follow-Up Biopsy Cases With HPV Co-Testing

Haijun Zhou, Roxanne Mody, Eric Luna, Donna Armylagos, Mary Schwartz, Dina Mody, Yimin Ge. Houston Methodist Hospital, Houston, TX; University of Texas, Health Science Center, Houston, TX; BioReference Laboratories, Houston, TX.

Background: High risk HPV (HR-HPV) testing for ASC-US triage and co-testing with cytology has been implemented in clinical practice for many years. Recently, the Cobas HPV test was approved by the FDA as an option for primary cervical cancer screening in women over 25 years. However, clinical data of primary screening using the HPV test alone in the routine practice setting for detecting HSIL is currently lacking.

Design: Of 130,648 Pap tests performed at Houston BioReference Laboratories and Houston Methodist Hospital between March 1, 2013 and June 30, 2014, 51,315 had Cobas HPV co-testing or reflex for ASC-US. Of these 51,315 cases, 1,654 had follow-up biopsies. The cytologic and biopsy interpretations were rendered by board-certified cytopathologists or gynecologic pathologists at an academic medical center. All biopsies with interpretation of CIN2 were confirmed with p16/Ki-67 immunohistochemical stains. The HSIL reporting rate in this general screening population was 0.24% with an overall cytohistologic correlation rate of 70%. The majority of non-correlations (98%) were tissue sampling variances.

Results: In 1,654 cases with follow-up biopsies, the sensitivities of Cobas HPV test and Pap test to detect any dysplasia are 80.7% and 81.2%, respectively and the positive predictive values are 69.1% and 69.2%, respectively. For biopsy-confirmed high-grade cervical lesions (CIN2/3, AIS or Carcinoma, n=245), the negative rates of the Cobas HPV test were 9.4% and the cytology negative (NILM) diagnoses were 9.0%. Co-testing with cytology and the Cobas HPV test only missed 4 (1.6%) biopsy-confirmed CIN2+ cases.

Conclusions: In our study cohort, the Cobas HPV test alone is not superior to Pap test as a primary screening method for cervical dysplasia. The false-negative rate of the Cobas HPV test alone in detecting biopsy-confirmed high-grade cervical lesions is comparable to that of the Pap test. Co-testing with the Cobas HPV test and Pap test is the best strategy in detecting high-grade cervical lesions.

Dermatopathology

448 Dermal Malignant Peripheral Nerve Sheath Tumors Have a Distinctive Profile af DNA Copy Number Changes From Melanoma

Aleodor Andea, Min Wang, Gina Johnson, Rajiv Patel. University of Michigan, Ann Arbor, MI; Emory University, Atlanta, GA.

Background: Histological and immunophenotypic distinction of superficial dermal malignant peripheral nerve sheath tumors (MPNSTs) from desmoplastic melanomas lacking a junctional component is a notoriously difficult diagnostic distinction. Our aim in this study was to evaluate the spectrum of copy number variations (CNVs) and allelic imbalances in a series of dermal MPNSTs and compare them to published data in melanomas in a attempt to establish a differentiating profile.

Design: Stringent criteria were used to select unequivocal examples of dermal MPNSTs: the tumor had to arise in association with a nerve, in a patient with neurofibromatosis type I and lack a junctional melanocytic component. Three such cases of dermal MPNSTs were identified for the study. Following DNA extraction we performed comparative genomic hybridization using the Oncoscan V3 platform. Data was analyzed with Nexus Express for OncoScan software.

Results: Results are shows in table (* denotes CNVs shared with melanoma). All cases showed genomic loss involving the NF1 gene. One case had no additional abnormalities while the other two showed a complex karyotype. With the exception of losses on chromosomes 9, 10 and 13, the other abnormalities encountered are not commonly described in melanoma. Frequently occurring CNVs in melanoma such as gains of 1p12-31, 4q12-13.1, 5p, 6p, 7, 8q and 11q and losses of 6q and 17p were not detected.

Case	Gains	Losses	Copy Neutral LOH
1	1q21.1-44*, 9p11.2-q34.3, 16q21-24.3, 17q23.2-25.3	3p26.3-12.1, 4q34.3-35.2, 8p23.3-p11.1, 9p21.3*, 9p24.3-23, 9p21.2-13.2, 10p15.3-q11.21*, 11p15.4-11.12, 13q21.31-21.33*, 17q11.2-21.32, 20p13-p11.21, 21q21.1-22.3	7q11.1-11.21, 16q11.2-24.3
2	-	17q11.2	-
3	17q21.32-25.2, 22q11.1-13.1	1p36.33-31.3*, 2q24.1-37.3, 3p11.1-26.32, 7q11.22-22.1, 8p23.3-11.21, 9p24.3-21.1*, 11.15.4-11.12, 16p13.3-p11.1, 17q11.2, 20q12-13.12, 22q13.31-13.33	17q11.2-21.32

Conclusions: Our data suggests that while there is some overlap, the spectrum of CNVs in dermal MPNSTs is more akin to deeply seated MPNSTs and distinctive enough to allow separation from melanoma. Evaluation of CNVs in these lesions may have role as a diagnostic tool.

449 Evaluation of a New Gene Expression Assay Aimed To Differentiate Nevi From Melanomas

David Arps, Katherine Brick, Min Wang, May Chan, Rajiv Patel, Aleodor Andea. University of Michigan, Ann Arbor, MI.

Background: There are subsets of melanocytic lesions in which conventional histologic criteria fail to reliably differentiate nevi from melanomas. Current ancillary molecular studies aimed to help in the diagnosis of ambiguous melanocytic lesions are based on assessing DNA copy number alteration either by comparative genomic hybridization