found between the number of positive biopsies and mortality. Only 6 of 42 diffuse C4d+ cases evolved to show DSA and dysfunction. Thirty six of 42 diffuse C4d+ cases showed no dysfunction, despite the presence of DSA in 6 of them. Thus interpreted as accommodation.
Conclusions: Serial staining of biopsies shows that: 1. Concomitant C4d and C3d positivity correlates highly with allograft dysfunction and DSA; 2. Diffuse C4d capillary staining alone should not be equated with AMR; 3. Most C4d+ episodes are single occurrences and asymptomatic; 4. The presence of C4d staining and DSA without allograft dysfunction may indicate accommodation; 5. Only a small fraction of patients with C4d staining alone may develop AMR on follow-up.

327 Carbonic Anhydrase IX – Hypoxia Marker in the Aortic Wall

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Background: Carbonic anhydrases (CA) catalyze conversion of CO_2 and H_2O to HCO_3^- and H^+. CA IX is particularly interesting because of its over expression in human cancers, likely to protect the tumor from acidotic environment engendered by anaerobic metabolism. Its expression is triggered by hypoxia-inducible factor-1 alpha, the master regulator of cellular response to hypoxia. Anti-CA IX antibody is clinically available for diagnostic imaging and potential cancer immunoradiotherapy.
Hypoxia in the vascular wall is one of the factors contributing to development of atherosclerosis and aortic aneurysms. Large arteries are particularly susceptible to hypoxia by nature of their blood supply. Luminal diffusion and adventitial vasa vasorum provide adequate vascular wall oxygenation in a healthy vessel, but fail to do so in various pathologic states. Indeed, frank aortic infarction is commonly seen in dissecting aortic aneurysms, following rather than preceding the latter (Circulation 1978;58:876-81).
Design: Using immunohistochemistry, we investigated expression of CA IX in aortic specimens obtained from patients with non-dissecting aortic aneurysms (n=13), dissecting aortic aneurysms (n=6) and granulomatous vasculitis (n=4). These were non-selected vascular surgical specimens received by our department within an 8-month period.
Results: In non-dissecting aneurysms, staining was frequently found in smooth muscle cells in the central media. In dissecting aneurysms, smooth muscle cells surrounding the areas of dissection were positive for CA IX. Vasculitis specimens exhibited strong CA IX staining within inflammatory foci and the surrounding smooth muscle cells. Staining was also seen in smooth muscle cells underlying atherosclerotic plaques and within the infarcted zones (CA IX is relatively resistant to proteolytic degradation). A further confirmation that CA IX staining truly indicates hypoxia was that prostate, testis and kidney infarcts (n=5) showed intense staining in zones bordering infarcted areas in which hypoxia would be anticipated.
Conclusions: These findings show that CA IX is consistently expressed in the diseased aortic wall and likely reflects hypoxic injury. CA IX up-regulation in the vascular wall is likely an adaptive mechanism aimed at preservation of tissue viability during hypoxic stress. A potential exists for in vivo assessment of CA IX expression in aortic wall for identification of past or on-going hypoxia.

328 The Changing Face of Infective Endocarditis: Ten Years Experience

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Background: Infective endocarditis (IE) has high morbidity and mortality. Over the years, there has been a shift in predisposing conditions, causative organisms, guidelines for prophylaxis, and treatment. For example, routine prophylaxis for dental procedures is no longer recommended in cardiac conditions which have lower risk of adverse outcome from endocarditis, such as mitral valve prolapse and aortic stenosis.
Design: Eighty-four cases of IE requiring surgical intervention were identified from the surgical pathology database over the past ten years. Clinical and pathologic features were obtained from the medical record and pathology reports, including demographics, source of infection and predisposing factors, disease course, and surgical treatment.
Results: Seventy-one cases of native valve, along with 13 cases of prosthetic valve IE were examined. In the native valve cases, the median age was 53 years and 64.8% were men. Of these cases, 24% were temporally associated with dental procedures, or occurred in patients with poor dentition; 18.6% of the cases occurred in patients with diabetes mellitus (DM), and 17.1% in patients with end stage renal disease. Only 5.7% of this patient population had infections related to intravenous drug abuse. The majority of the causative organisms were oral and skin flora, consisting of Strep viridans (28.6%) and coagulase negative Staph (11.4%). Methicillin resistant Staph aureus (MRSA) was the pathogen in 17.1% of cases. In 51.3% of the cases, an underlying native valve or heart disease was identified as a risk factor for the infection, of which 15% were mitral valve prolapse and 15% bicuspid aortic valve. Only one case of underlying rheumatic heart disease was identified. All valves showed acute and chronic changes, 91.4% had vegetations noted by imaging or pathologic examination, and 73.2% had organisms seen histologically in spite of antibiotic therapy. There was a trend towards more valve repairs, as opposed to replacement, in more recent years.
Conclusions: In this contemporary series of IE cases undergoing surgery, many more cases are associated with mitral valve prolapse or bicuspid aortic valve than rheumatic heart disease. Many patients had DM and renal failure predisposing to infection. Oral and skin flora were the causative organisms in the majority of cases; however MRSA was frequently identified. In spite of recommendations for more restricted use of prophylaxis, in this series, there was continued association between dental procedures/disease and endocarditis.

329 Pathological Features of Adventitial inflammatory Reaction in Acute Aortic Dissections

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Background: Histological reaction in the adventitia to aortic dissections has not been well studied. We present histological findings in a series of acute aortic dissections with emphasis regarding dating and inflammatory reaction.
Design: We prospectively studied 43 surgically excised acute ascending aortic dissections. We evaluated the histological reaction in the media and adventitia adjacent to the acute dissection plane in 4 or more sections of aorta oriented perpendicularly. Inflammation (both degree and type) and stromal reaction were semiquantitated and correlated with duration of symptoms prior to surgical repair.
Results: Of the 43 cases, there were 31 men (ages 53 ± 14 years) and 13 women (ages 60 ± 17 years). Duration of symptoms was classified as <12 hours (n= 9), 12-24 hours (n=12), 1-2 days (n=8), 2-7 days (n=11), and > 1 week (n=3). Medial inflammation was usually relatively sparse. When present, neutrophils were detected within 12 hours, and in one case there was intense medial inflammation suggestive of aortitis. Lymphocytes were present after 12 hours, and macrophages were present after 1 day and peaked between 2-7 days. Lymphocytes and macrophages were observed in 80% of cases occurring in this time frame, and were numerous in 3 cases mimicking vasculitis. Comparatively, adventitial inflammation was relatively brisk. Neutrophils occurred before 12 hours, peaked between 12-24 hours, and were rare after 2 days. Eosinophils occurred after 1 day, peaked between 2-7 days, and were predominant in 3 cases between 2-7 days. Apoptosis occurred after 12 hours, peaked between 1-2 days; mitotic figures were present in similar time frame as apoptosis, but were still numerous up to 7 days. Macrophages followed by reactive fibroblasts were present after 1 day and peaked 2-7 days. Prominent inflammation of adventitial nerves and proliferation of paraganglial cells were observed in 7 cases; these cases showed neural influx of atypical macrophages and stromal cells. Hemosiderin-laden macrophages were present only in one case, which had a concomitant healed dissection.
Conclusions: Reactive changes in the adventitia and media are fairly reliable, and can be used to date aortic dissections in the first week after medial rupture. Inflammatory reaction in the media can occasionally contain numerous neutrophils, lymphocytes, or macrophages, mimicking vasculitis, and eosinophils may be prominent in the adventitia.

Cytopathology

330 The Utility of Fine-Needle Aspiration in the Diagnosis of Primary Lung Tumors and Metastatic Tumors to the Lung, a Retrospective Examination of 1032 Cases

JA Adams, HH Wu. Indiana University School of Medicine, Indianapolis, IN.
Background: With the emergence of improved treatment strategies for patients with malignant lung tumors it has become increasingly more important to adequately diagnose and subclassify lung lesions. In our large retrospective study, we assessed the utility of fine needle aspiration (FNA) in the diagnosis of primary and metastatic tumors to the lung.
Design: We reviewed the archived reports for 1032 patients undergoing FNA of primary lung tumors, metastatic lung tumors, and metastatic tumors to the lung. Based on the diagnoses that were rendered, the cases were grouped into atypical, benign, malignant, nondiagnostic, and suspicious lesions. The malignant FNA cases were further subclassified based on tumor type. Cases with correlating histology were then reviewed and diagnoses compared.
Results: The 1032 FNA cases were grouped as follows; 34 (3.3%) atypical, 142 (13.8%) benign, 717 (69.5%) malignant, 121 (11.7%) nondiagnostic, and 18 (1.7%) suspicious. Subclassification of malignancies diagnosed on FNA were as follows; 297 (41.4%) adenocarcinoma, 159 (22.1%) squamous cell carcinoma, 56 (7.8%) small cell carcinoma, 53 (7.4%) non-small cell carcinoma (NSCLC), 123 (17.2%) metastatic tumors, 15 (2.1%) neuroendocrine carcinoma, and 7 (1%) poorly differentiated carcinoma. Out of all NSCLC cases, 90% were able to be subclassified into either adenocarcinoma or squamous carcinoma on cytomorphology alone or with the help of immunohistochemical stains. Immuno stains were performed on 276 (27%) of the cases. The most frequent origins of metastatic tumors were renal cell carcinoma (22), melanoma (17), colon (15), breast (14), and urothelial carcinoma (10). There was also metastasis from 20 other organs with fewer than 4 cases each. 196 of 335 histologic follow-up specimens were biopsies (transbronchial or transthoracic core). Comparison of the FNA and surgical biopsy showed a sensitivity of 96% for FNA versus 98% for biopsy and a specificity of 100% for both. Sampling error resulted in 8 false negative cases on FNA. The diagnostic rate for FNA was 88.3% (vs 96% for surgical biopsy) and 91.6% of FNAs were able to specifically subtype a malignancy compared to only 80.6% of surgical biopsies.
Conclusions: FNA is comparable to histologic examination in the diagnosis and subclassification of both primary and metastatic lung tumors. 90% of NSCLC cases were able to be further subclassified into adenocarcinoma or squamous cell carcinoma by FNA.

331 Thyroid Bed Fine-Needle Aspiration: A Clinicocytologic Correlation

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Background: Monitoring changes in the thyroid bed (TB) is one of the clinical mainstays for surveillance of recurrent thyroid carcinoma. Fine needle aspiration (FNA) is a diagnostic tool that is commonly used to aid in the decision of further clinical treatment options and follow-up.

Design: We retrieved cases of soft tissue masses within the thyroid bed that were evaluated for recurrence between January 1, 2006 and February 1, 2011. All ultrasound-guided biopsies clinically suspected to be lymph node metastasis or had lymphocytes present on the FNA were excluded and only one FNA from each patient was included. If multiple biopsies were performed, only the positive FNA was included.

Results: 292 patients were identified for evaluation of recurrence; 250 papillary thyroid carcinoma (PTC), 15 follicular carcinoma, 21 medullary carcinoma, and 6 Hurthle cell carcinoma. For all FNAs that were clinically suspicious for recurrence the rate of positivity was 66.1% (193 patients). 14 of the 69 patients (20.3%) diagnosed with a negative FNA proceeded on to surgical resection or ethanol ablation. The average time between thyroidectomy and thyroid bed FNA was 76 months. On the subset of patients with a previous diagnosis PTC, who were diagnosed with either suspicious or positive for recurrent PTC on TBFNA; the following clinicopathologic parameters were found to be statistically significant.

P-values for Papillary Thyroid Carcinoma and Associated Risk Factors

Parameter	Other (negative, non-diagnostic, atypical)	Suspicious/ Positive	Total	p-value***
	(N=69)	(N=179)	(N=248)	
Sex				0.0188
Female	56 (81.2%)	118 (65.9%)	174 (70.2%)	
Male	13 (18.8%)	61 (34.1%)	74 (29.8%)	
Mean (SD) Tumor size	1.9cm (1.5)	2.7 cm (1.4)	2.4 cm (1.5)	<0.0001
Metastases				<0.0001
Absent	31 (44.9%)	21 (11.7%)	52 (21.0%))	
*Present	38 (55.1%)	158 (88.3%)	196 (79.0%)	
Extrathyroidal extension				0.0024
Unknown or confined	53 (76.8%)	103 (57.5%)	156 (62.9%)	
Present	16 (23.2%)	76 (42.5%)	92 (37.1%)	

*Includes lymph node and all other metastases. ***From chi-square test or Fishers exact test for categorical data; Wilcoxon rank-sum test for continuous data.

Age at thyroidectomy and number of cancer foci were not found to be statistically significant.

Conclusions: Thyroid bed recurrence of PTC is most likely to occur in patients who have the following clinicopatholgic parameters: female sex, documented metastasis to any site, extrathyroidal extension, radioactive iodine treatment prior to FNA and larger primary tumor size.

332 Endoscopic Ultrasound Guided Fine Needle Aspiration as a Diagnostic and Staging Tool for Rectal and Perirectal Lesions – An Institutional Experience

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Background: The role of endoscopic ultrasound guided fine-needle aspiration (EUS-FNA) in evaluating lesions adjacent to the upper gastrointestinal tract wall is well established. However, this tool is under-utilized in evaluating rectal and perirectal lesions, possibly due to insufficient experience and under recognized value of this procedure. In this study, we report our institutional experience with EUS-FNA as a diagnostic and staging tool for rectal and peri-rectal lesions.

Design: A retrospective chart review was performed and a cohort of 36 patients who underwent rectal EUS-FNA (39 specimens) at our institution between January 2002-July 2011 was retrieved. The cytology diagnoses were compared to the concurrent or follow up histologic and clinical diagnoses.

Results: Among the total 39 cases, rectal EUS-FNA was performed as a diagnostic procedure in 21 (54%) and a staging procedure in 18 (46%) cases. On cytology examination, 15 (39%) cases were diagnosed as malignant, 1 (2%) as atypical/suspicious for malignancy, 3 (8%) as benign neoplastic, 14 (36%) as benign reactive and 6 (15%) as non-diagnostic. Malignant cases included 11 colorectal/anal, 1 cervical, 1 endometrial, 1 urothelial, 1 hematopoietic and 1 unknown primary cancers. Concurrent or follow-up histological diagnoses were available in 19 (48%) cases, 18 of them had concordant cytological/histological diagnoses (10 benign, 8 malignant). One perirectal lymph node with negative cytology diagnosis was found to be positive on histologic examination, probably due to sampling error on cytology. The sensitivity and specificity of EUS-FNA for rectal/perirectal lesions in this study was 90% and 100% respectively.

Conclusions: EUS-FNA is a useful diagnostic tool for rectal/perirectal lesions; it confirms or excludes malignancy for lesions with high or low clinical suspicions. It serves as a reliable staging method to identify patients for proper clinical management. The prevailing non-diagnostic rate may be further reduced as more experience is gained with this procedure.

333 Atypia of Undetermined Significance: The Thyroid FNA Experience at University Hospital in San Antonio

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Background: According to the recently proposed Bethesda System for Reporting Thyroid Cytopathology (TBSRTC), the category of Atypia of Undetermined Significance (AUS) is reserved for specimens that contain cells with architectural and/or nuclear atypia insufficient to be classified as suspicious or malignant but the atypia cannot be confidently called benign. The recommendation is to use this category as a last resort and limit its use to approximately 7% or fewer of all thyroid fine needle aspirations (FNA). Since its publication in 2009, several institutes have published different percentages of reporting AUS. Also the follow up and outcome data in this category is limited. The objective of this study was to report our experience with the AUS category, to correlate these cases with the results of repeat cytology or surgical resection and to evaluate whether a repeat FNA versus surgical resection as follow-up would be a better option.

Design: A computerized search for all thyroid FNAs was performed from January 2008 to September 2011. For the FNA cases identified from Jan 2008 to December 2009, three cytopathologists who were blinded to the original diagnosis, re-classified the diagnosis using the TBSRTC. Starting January 2010 up to present, our institution implemented the TBSRTC. All cases which fell under the AUS category were selected and correlated with follow-up cytology or surgical specimen.

Results: Of a total of 985 thyroid FNAs reported, 86 (8%) had a diagnosis of AUS. Only 13/86 (15%) had follow-up repeat cytology (non-diagnostic 2, benign 8, AUS 2, malignant 1). Forty cases (46%) had surgical follow-up with the following results: Benign 32, Follicular lesion of uncertain malignant behavior 1, Follicular carcinoma 1, Papillary Carcinoma 6.

Conclusions: The proposed Bethesda algorithm for clinical follow-up of patient with an initial diagnosis of AUS is repeat FNA within 3-6 months, preferably ultrasound guided, and subsequent surgical resection if the follow-up FNA is AUS or worse. However, compressive symptoms or worrisome ultrasound findings frequently led to resection in lieu of repeat FNA. Given that 61% had a benign diagnosis on repeat FNA and 80% had a benign diagnosis on surgical resection, a repeat FNA is a reasonable option as opposed to surgical resection, which carries with it, associated morbidity.

334 Accuracy and False-Positive Rate of the Cytologic Diagnosis of Follicular Cervicitis: Observations from the College of American Pathologists Pap Educational Program

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Background: Follicular cervicitis has been viewed traditionally as a relatively simple cytological diagnosis. There are, however, occasional cases of follicular cervicitis, reported in the literature, that have been misinterpreted, leading to false-positive diagnoses. The objective of this study was to determine the accuracy of the diagnosis and the false-positive rate of follicular cervicitis in gynecologic cytology by assessing the responses of participants in the context of the College of American Pathologists (CAP) PAP educational program.

Design: We performed a retrospective review of 4914 participant responses for gynecologic cytology challenges with the reference diagnosis of follicular cervicitis from 2000 to 2010 from the CAP PAP educational program. Reference diagnosis category, false-positive rates by participant type, and preparation type (conventional smears versus ThinPreps) were analyzed.

Results: Of the total 4914 general category responses, 4368 (88.9%) were benign while 546 (11.1%) responses were abnormal (>/= LSIL); the latter correspond to the false-positive diagnoses. Of the benign responses, only 2026 (46.4% of the benign responses) were an exact match to follicular cervicits. Adenocarcinoma and HSIL were the most common diagnoses chosen in the false-positive interpretations, accounted for 42.3% and 20.1% of the false-positive diagnoses respectively. Reader type was significantly associated with false positive diagnoses (laboratory: 19.2% versus cytotechnologist:11.1% versus pathologist: 7.9%; p<.001). Thin-Prep was also significantly associated with false-positive diagnoses as compared to conventional smears (12.2% versus 3.6%; p<.001).

Conclusions: In an education program, follicular cervicitis is difficult to diagnose accurately and represents an important cause of false-positive responses in gynecologic cytology. These results highlight the importance of follicular cervicitis as a mimic of adenocarcinoma and HSIL, in particular in ThinPreps.

335 Reproducibility Assessment of Hormonal Receptor Status and HER2 in Cytology Specimens by Image Analysis: A Pilot Study

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Background: Estrogen receptor (ER), Progesterone receptor (PR) and HER2 status evaluation in primary and metastatic breast cancer in cytology specimens has known prognostic and therapeutic implications. The aim of this study is to assess the concordance of ER/PR/Her2 results in cytology specimens by recently implemented in our laboratory Image Analyzer (Ventana, Tucson, AZ) and semiquantitative scoring by pathologists. To our knowledge, this is the first study of this kind.

Design: Twenty three consecutive ER/PR/HER2 immunostained cytology specimen cell block preparation slides from primary and metastatic breast carcinoma for the years 2009-2011 were retrieved, blindly reviewed by 5 cytopathologists, and scored per CAP/ASCO guidelines. The Image Analyzer (IA) results of these cases were retrieved and the scores compared with blind review (BR). ER/PR/HER2 status of metastatic tumors was also compared with that of primary tumor (PT) when available. Fluorescence in situ hybridization (FISH) results of equivocal HER2 cases were documented.

Results: Out of 23 cases (7 FNA from metastatic lesions and 16 effusion fluids), 1 was insufficient for analysis. Four cases were discordant between the IA and BR scores. Two cases showed discordance in the Her2 analysis: IA: 2+, BR: 1+. Her-2/neu was not amplified by FISH in both cases. In 2 other cases, IA picked up weak positivity in PR which was missed by BR.

The ER/PR/Her2 status of PT was available in 20 cases. Discordance between the PT and the IA scores was seen in 8 cases. In 4 cases, IA scored the Her2 as 2+, while the Her2 in PT was negative. Her2 was not amplified by FISH in these cases. In 2 cases of metastatic effusions, IA scored ER as positive and weakly positive, while in PT they

were reported as negative. In 1 case of metastatic effusion, PR was scored as positive while PT was negative. Lastly, in 1 case of metastatic effusion, PR was scored as negative while PT was reported as positive.

Conclusions: IA appears to be more sensitive in assessing weak hormone receptor positivity than BR. One of the frequent discrepancies between both comparative groups (IA vs BR and IA versus PT) was the evaluation of Her2 score. It appeared to be overcalled by IA. Discrepant cases between IA and PT status may represent transformation of the receptor status, inadequate sampling, or limitations due to scant cellularity in cell blocks. Overall, IA appears to be an efficient method for evaluation of ER/PR/HER2 status in cytology specimens; however, larger studies should be done to assess accuracy of this method in cytology samples.

336 Atypia in Papillary Lesions of Breast in Fine Needle Aspiration Biopsy and Association with Malignancy

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Background: Fine needle aspiration biopsy(FNAB) is a frequiently used method for diagnosing papillary lesions of the breast. The challenge is in differentiating papillomas from benign non papillary proliferations and to distinguish benign papillomas from malignancy which includes papillary and non papillary types.

Design: The pathology database of Beth Israel medical center was searched for women who under went excisional biopsy after an initial diagnosis of papillary lesion(PL) on FNAB. 130 cases were identified. The biopsies were done with and with out ultrasouind guidance.Ages ranged from 20 to 92. The findings were categorised on the basis of presence or absence of atypia on FNAB and the final diagnosis.

Results are shown in the tables.

Results:

Diagnosis on excisonal biopsy	PL with atypia on FNAB n (%)	PL without atypia on FNAB n (%)
Carcinoma(IDCA, DCIS, Papillary) n=28	12 (9.2%)	16 (12.3%)
LCIS n=7	3 (2.3%)	4 (3%)
Papilloma with atypia n=6	4 (3%)	2 (1.5%)
Papilloma n=63	8 (6.1%)	55 (42.3%)
FCC/FA/BPT n=25	0 (0%)	25 (19.2%)
Adenomyoepithelioma n=1	0 (0%)	1 (0.7%)

IDCA- Invasive duct carcinoma, DCIS- Ductal carcinoma insitu, LCIS- Lobular carcinoma insitu, FCC- fibrocystic change, FA- fibroadenoma, BPT- Benign phyllodes tumor

Conclusions: Absence of atypia in FNAB did not exclude malignancy.

Presence of atypia in FNAB had a high incidence of carcinoma on excisional biopsy.

Presence of atypia was not always associated with malignancy.

High incidence of carcinoma warrants surgical excision of papillary lesions for further evaluation.

337 The Bethesda System for Reporting Thyroid Cytopathology: A Meta-Analysis

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Background: The Bethesda system for reporting thyroid cytopathology (TBSRTC) represents a further step toward standardization of reporting thyroid cytopathology and is now well established in the US. Through meta-analysis, we aim to investigate the validity of using the 6-tiered reporting system in US and European laboratories.

Design: All publications up to 01.09.2011 dealing with TBSRTC and with available histological follow-up were retrieved. To calculate sensitivity, specificity, diagnostic accuracy, we defined the true-positive as the follicular neoplasm+suspicious of malignancy+malignant FNA specimens that were histopathologically confirmed as malignant; the true-negative as the benign FNA specimens that were histopathologically confirmed as benign; the false-positive as the follicular neoplasm+suspicious of malignancy+malignant FNA specimens that were histopathologically confirmed as benign; and the false-negative as the benign FNA specimens that were histopathologically confirmed as malignant. Non-diagnostic and atypia of undetermined significance categories were excluded from the statistical calculation. Correlation between the six diagnostic categories were performed.

Results: The case cohort included a total of 24,445 thyroid FNAs, of which 5911 (24.2%) were followed-up by surgery and constituted the basis of the study. Cyto-histological correlations are showed in Table 1.

Cyto-histological correlations of TBSRTC

Cytological diagnosis	**All FNAs**	**All FNAs with histological follow up**			**Benign histology**	**Malignant histology**
	n (%)	**n**	**% total (a)**	**% category (b)**	**n (%)**	**n (%)**
Non-Diagnostic	3215 (13.1)	511	8.6	15.9	427 (83.6)	84 (14.4)
Benign	14433 (59.0)	1390	23.5	9.6	1344 (96.7)	46 (3.3)
Atypia of undetermined significance (AUS)	2433 (10.0)	949	16.1	39.0	798 (84.1)	151 (15.9)
Follicular neoplasm (FN)	2399 (9.8)	1631	27.6	68.0	1196 (73.3)	435 (26.7)
Suspicious for Malignancy	656 (2.7)	477	8.1	72.7	114 (23.9)	363 (76.1)
Malignant	1309 (5.4)	953	16.1	72.8	12 (1.3)	941 (98.7)
Total	24445 (100)	5911	100	24.2	3891 (65.8)	2020 (34.2)

(a) Percentage of the total 5911 operated cases; (b) Percentage of cases operated in each diagnostic category.

Sensibility, specificity and diagnostic accuracy were 97.4%, 50.4% and 69.3%, respectively. There were strong correlations noted between the diagnostic categories of the 6-tiered system and the histological follow-up data in predicting outcome.

Conclusions: TBSRTC represents a valuable reporting system based upon results of meta analysis showing high overall accuracy.

338 FISH and *KRAS* Mutation Testing as Adjuncts to Biliary Brushing Cytology for the Detection of Pancreatobiliary Tract Malignancy

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Background: ERCP with biliary brushing cytology is routinely performed for the evaluation of patients with biliary tract strictures. Although the specificity of this approach in the detection of carcinoma is nearly 100%, sensitivity rates have varied widely. Recent studies suggest molecular techniques may play a useful role in increasing the sensitivity of biliary brush cytology. The purpose of the current study was to examine the clinical value and performance characteristics of adjunctive studies to include *KRAS* mutation and fluorescence in situ hybridization (FISH) analysis at our institution.

Design: Based on a high clinical suspicion for malignancy, 19 specimens from 17 patients (6 female/11 male; 42-81 yrs of age) were selected for ERCP, cytologic examination, *KRAS* mutation testing by pyrosequencing, and copy number assessment of chromosomal loci 3, 7, 9p21, and 17 using the UroVysion FISH probe set (Abbott Laboratories). A specimen was classified as abnormal if five or more cells demonstrated polysomy; polysomy defined as gain of two or more fluorescently labeled probes to the centromeres of chromosomes 3, 7, and 17 and chromosomal band 9p21 in a single cell.

Results: All cases were negative for *KRAS* mutations. All 6 cases with abnormal cytologic and/or histologic findings were FISH positive (Table 1); adenocarcinoma was diagnosed in 2/6 patients following subsequent additional sampling (lymph node). A single FISH positive patient with negative cytology/histology had an elevated CA19.9 with only 2 wks clinical follow-up.

Table 1: Results (# of specimens)

FISH positive (7)								**FISH** negative (9)							
[1]*pathologic dx* atypical/suspicious (6)				[1]*pathologic dx* negative (1)				[1]*pathologic dx* atypical/suspicious (0)				[1]*pathologic dx* negative (9)			
[2]CA19.9 + (4)		[2]CA19.9 – (0)		[2]CA19.9 + (1)		[2]CA19.9 - (0)		[2]CA19.9 + (0)		[2]CA19.9 - (0)		[2]CA19.9 + (5)		[2]CA19.9 - (3)	
PSC + (0)	PSC- (4)	[3]PSC+ (1)	PSC- (0)	PSC+ (0)	PSC- (1)	PSC+ (0)	PSC- (0)	PSC+ (0)	PSC- (0)	PSC+ (0)	PSC- (0)	PSC+ (2)	PSC- (3)	PSC+ (1)	PSC- (2)

[1]Pathologic dx (diagnosis) includes biliary brushing cytology and corresponding biopsy results.
[2]CA19.9 analysis was not conducted on all patients.
[3]This PSC patient did not have CA19.9 testing.

Conclusions: These data confirm the clinical utility of FISH as an adjunct to routine bile duct brushing cytology in increasing the detection of pancreatobiliary tract malignancy in patients with biliary stricture. In contrast, our *KRAS* findings differ from previous reports that have suggested *KRAS* testing in biliary brushing specimens increases cancer detection rate. However, the latter discordance could be explained at least in part by employment of different methodologic approaches with distinct mutation detection sensitivities. Interestingly, one FISH positive patient was diagnosed with biliary papillomatosis, an entity not previously subjected to molecular cytogenetic characterization.

339 HSIL Is as Elusive on ThinPrep Paps as on Conventional Paps

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Background: 68.6% of *Conventional* Pap Smears immediately preceding a histological diagnosis of HSIL (Index Pap) show "minor" abnormalities (ASC-US, AGC and LSIL) per *Obstet Gynecol;1998;91:973.* Corresponding data for liquid-based Pap test (ThinPrep) are scarce.

Design: All ThinPrep Pap Tests immediately preceding biopsy-proven HSIL (Index TPPT) for 4 years, 2007-2010, were evaluated. Index TPPT with "major" abnormalities (HSIL, ASC-H, LSIL-H) were considered concordant, and those with "minor" abnormalities (ASC-US, AGC, LSIL) or negative were considered discordant. Available Index TPPT were reviewed to determine causes for discrepancy.

Results: 493 patients, each with one biopsy diagnosis of HSIL (age range: 14-78 years; mean: 33; <30: 231, >30:262). 274/493 (55.5%) of Index TPPT were discordant (including 24 negative cases), and 219/493 (44.4%) were concordant (Table 1). Endocervical cells and/or transformation zone (EC/TZ) was represented in 202/219 (92.2%) concordant and 215/274 (78.4%) discordant cases (P-value<0.0001). CIN2 was histologically diagnosed in 111/219 (51%) concordant cases and 186/274 (68%) discordant cases (P value=0.0001). hr-HPV test was positive in 93% of Index TPPT (wherein tested: 365/392). 233/274 discordant index TPPT were available for review: 231/233 (99%) did not show HSIL (true-negative, TN), and 2/233 (1%) showed HSIL (false-negative, FN). Each of the two FN cases preceded histological diagnosis of HSIL by <6 weeks, and each lacked classical HSIL cytomorphology and were misinterpreted as squamous metaplasia. Main causes for discrepancy in the 231 TN Index TPPT were: clinical sampling (187/231) including 59 cases which lacked EC/TZ, excessive cytolysis (34/231) and scant squamous cellularity (10/231).

ThinPrep Pap Test Abnormalities in Histologically Diagnosed HSIL in 493 Patients

HISTOLOGY	ASC-US	ASC-H	AGC	LSIL	LSIL-H	HSIL	TOTAL
CIN 2	101	23	1	71	42	46	297
CIN 2-3	27	8	0	19	11	31	102
CIN 3	15	9	5	11	6	43	94
TOTAL	143	40	6	101	59	120	493

Conclusions: 55.5% of Index ThinPrep Paps immediately preceding a histological diagnosis of HSIL were either negative or showed only "minor" abnormalities. Discordant cases were significantly associated with absence of EC/TZ on ThinPrep Pap and with CIN2 on biopsy. In this series, hr-HPV test was more predictive of HSIL than the ThinPrep Pap test. In this setting, causes of cytological-histological discrepancies deserve additional study.

340 Reporting Thyroid FNA before and after Implementation of Bethesda System

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Background: *The Bethesda System for Reporting Thyroid Cytopathology* was published in 2008, and was implemented at Beth Israel Deaconess Medical Center (BIDMC) in June 2010. Prior to this date, our diagnostic scheme was similar to the Bethesda System, except for the category of "Atypia/Follicular Lesion of Undetermined Significance" (AUS). Instead, we used the categories "Atypical (ATY)," "Suboptimal (SUB)" and "Indeterminate (IND)" to describe specimens that would later be classified as AUS. This study evaluates the impact of the Bethesda System on the rate and the positive predictive value (PPV) of the diagnostic categories at BIDMC.

Design: We performed a retrospective review of all thyroid FNAs during the time periods Jan 2006-Nov 2008 and June 2010-July 2011 (period 1 and 2, respectively) and identified 2355 and 1204 specimens, respectively. Each cytology report was categorized as shown in Table 1 below. All subsequent thyroidectomy specimens were identified (406 and 227 from period 1 and 2, respectively) and categorized as benign or malignant. PPV was determined for each cytologic category.

Results:

Table 1

Diagnostic Category	1st Period		2nd Period	
	% of total	PPV in %	% of total	PPV in %
Non-Diagnostic (ND)	13	15	17	23
ND-cyst			4.8	33
SUB-benign, including cysts	21	15		
SUB-Hürthle cell (HC) lesion or microfollicular lesion	2.5	17		
SUB-papillary carcinoma (PC)	0.93	36		
Benign	41	17	48	23
ATY/AUS	3.7	31	15	44
HC neoplasm	3.1	24	2.4	36
Follicular neoplasm	3.4	30	4.0	32
IND-PC	2.5	53		
Suspicious	3.9	87	4.5	80
Positive	4.5	98	4.2	100

The most notable change between the two periods was an increase during the second period of the rate of ND (13% to 22% [including cysts]), Benign (41% to 48%), and AUS (3.7% to 15%) diagnoses. These increases were a result of the inclusion of those cases previously classified as SUB and IND-PC into these categories in the new scheme. Consequently, an increase in PPV was seen for these three categories, while the PPV for the other categories remained relatively unchanged. This is especially evident in the PPV of the AUS category (44%), which includes previous diagnoses with PPVs ranging widely from 17% (SUB-HC) to 53% (IND-PC).

Conclusions: Implementation of the Bethesda System to yield recommended PPVs is highly lab-dependent. Diagnoses with wide-ranging PPVs are now grouped into the AUS category, which has a PPV approaching 50% in our lab; this creates uncertainty regarding the appropriate management for this category. Our prior scheme provided greater diagnostic specificity to direct clinical management of these patients.

341 Comparison of Estrogen Receptor Immunostaining in Papanicolaou-Stained Direct Smears and Matched Cell Block Sections

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Background: Cell block (CB) is the optimal cytologic specimen for evaluating estrogen receptor (ER) in patients with breast carcinoma. The need for ER immunostaining (IS) of Papanicolaou (Pap)-stained direct smears (DSs) arises in patients with breast carcinoma when CBs cannot be prepared because of limited cellularity of the aspirate. The primary objective of our study was to compare the IS of Pap-stained DSs with corresponding formalin-fixed and paraffin-embedded CB sections.

Design: The study included 68 matched specimens of Pap-stained DSs and CBs from 18 effusions with metastatic breast carcinoma and 16 scrapes of surgical specimens of breast carcinoma (34 cases). Immunostaining for ER was performed using a polymeric biotin-free horseradish peroxide (HRP) method on (Leica Microsystems, Bannockburn, IL) using anti- ER antibody (Clone ER 6F11, dilution 1:35, Novocastra) with antigen retrieval (citrate buffer). The proportion of ER nuclear positivity in the tumor cells was expressed as a percentage and categorized as low positive (LP) (1% to 9% ER +) or as positive (≥10% ER +); intensity was scored on a scale of 1-3. Comparison of the ER IS of DS and CB was performed using the kappa statistic.

Results: The DSs and CBs demonstrated positive ER IS in 24 (71%) DSs and 26 (76%) CBs, and were negative in 10 (29%) DSs and 8 (24 %) CBs. The DSs were positive for ER in 22 specimens and LP in 2, whereas the CBs were positive in 24 specimens and LP in 2. Except for one DS, the proportion and intensity of IS was higher in DSs than in CBs. There were concordant results for ER IS in 30 (88%) of the 34 cases. We found discrepant results in 4 cases (12%); 3 DSs with negative results where the CBs showed positive IS (20%, 1+; 10%, 2+; 50%, 1+) and 1 DS with low positive (1%, 1+) ER with negative results on CB. Comparison of DSs with CBs revealed a kappa statistic of 0.70, which indicates substantial agreement.

Conclusions: 1. Pap-stained DSs were comparable to CBs for ER IS.
2. The proportion and intensity of ER IS was higher in DSs than in CBs.
3. Discrepant cases mainly consisted of negative results in DSs corresponding to weakly ER-positive CBs.
4. Whereas DSs positive for ER expression indicate true positives, a negative result may represent either a true-negative or false-negative result.

342 Evaluation of Image Guided Core Biopsies by Touch-Prep: Utility and Limitations

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Background: Touch-Prep (TP) on image guided core biopsies has the potential to avoid non-diagnostic sampling and allow for immediate triage of specimens. This potential, in turn, is dependent upon the congruence in cytologic and histologic interpretations. We reviewed TP and core biopsy diagnoses and identified factors that are likely to cause discordant interpretations. In an era of increasing reliance on smaller specimens, effective triage and efficient utilization of material is crucial.

Design: We performed a one year (2010) retrospective analysis of all image guided core biopsies with TP. 214 cases were identified, of which 78 (36%) were lung, 40 (19%) liver and 96 (44%) other (lymph node, bone, mediastinum, intraabdominal, etc.). TP cases were categorized as positive, negative or atypical and compared to the histologic diagnoses. Discrepant cases were reviewed and the medical record was consulted for follow-up in cases without subsequent biopsy.

Results: See Tables 1 - 3.

Site	TP	Core Bx	Review comments of TP and core	Follow-up
Liver	Positive for malignancy. Non-small cell carcinoma	Cirrhosis	Limited number of malignant cells at one edge, a lot of fibrosis	Subsequent biopsy+ fibrotic tumor, clinically suspect cholangiocarcinoma
Lung	Rare non-small cell carcinoma.	Rare, atypical cells suspicious for non-small cell carcinoma on TP. No tumor seen in core biopsies.	Interstitial fibrosis	Lobectomy, Poorly differentiated adenocarcinoma with sarcomatoid features.
Lung	Necrosis with rare tumor cells, favor non-small cell carcinoma.	Extensive necrosis and fibrosis with rare atypical cells suspicious for non-small cell carcinoma.	Necrosis/fibrosis	Clinically widely metastatic NSCLC. Biopsy of duodenal mass + for non-small cell, thought to be a metastasis from lung
Lung	Non-small cell carcinoma	Rare markedly atypical cells highly suspicious for non-small cell malignancy	Scant cells on TP, core tiny	Clinical history of melanoma, and SCCA of tonsil, with a current brain metastasis and lung mass, no follow-up bx
Lung	Tumor present	Highly suspicious for adenocarcinoma	Fibrosis and necrosis	Stage IV lung cancer 3 years prior s/p chemotherapy, no follow-up bx
Pelvic mass	Adequate favor colorectal	Non-small cell carcinoma identified on TP. Core biopsy is non-diagnostic	Fibrosis and necrosis	Stage IV colon cancer, s/p therapy, no follow-up procedure due to comorbidity
Periaortic lymph node	Rare malignant cells in lymphoid stroma	Core biopsy quantitatively insufficient	Insufficient for comparison	Flow cytometry negative, 3 months prior, + pancreas FNA of poorly differentiated malignancy, no f/u bx
Lung	Positive for malignancy; non-small cell carcinoma.	NEOM. Chronic inflammation with marked fibrosis and organizing pneumonia with foci of acute inflammation	False positive attributed to reactive changes.	Follow-up mass resolved with antimicrobial therapy, no residual imaging abnormality

Table 1. Discordant cases with positive TP and negative/atypical/suspicious core biopsy.

Fourteen discordant cases (6.5%) were identified, of which 71% demonstrated mostly blood, necrosis/debris, crush artefact or degeneration. Of these 14 there were 4 false negative touch-preps.
Twenty-eight (13%) cases were called atypical on touch preparation. Twenty-two of these (79%) were malignant and 6 (21%) were benign on core biopsy. The overall trend was not site specific (lung 11, liver 7, other 10).
Forty-four cases were metastatic disease, 15 had diagnostic flow cytometry and 10 had useful immunohistochemistry.

Table 2. False negative TP, Atypical TP, and ancillary studies on TP

Overall n=214 (%)	BX+	BX-	Lung n=78	BX+	BX-	Liver n=40	BX+	BX-
TP+	100 (46)	1 (0.04)	TP+	30 (39)	1 (1)	TP+	14 (35)	0
TP-	4 (1.8)	71 (33)	TP-	1 (1.2)	29 (37)	TP-	1 (2.5)	12 (30)

Overall TP sensitivity 96%, specificity 98.6%, PPV 99%, NPV 94%

Table 3. Summary statistics

Conclusions: Touch-Prep on core biopsies is very accurate and contributes to a more efficient and effective use of very small specimens. Discordance between TP and core biopsy is often due to necrosis, obscuring blood or tumor characteristics such as marked fibrosis with few tumor cells. When correlated with the corresponding definitive specimen, TP identified more positive specimens than core biopsies. The presence of tumor cells in the TP should not be ignored when absent on the core biopsy. Until ancillary tests are adapted to cytologic material, cytopathologists should encourage radiologists to obtain sufficient viable and cellular material to perform such tests.

343 Endoscopic Ultrasound-Guided Fine Needle Aspiration (EUS-FNA) Biopsy of Solid Pancreatic Lesions: Review of 681 Cases

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Background: Endoscopic ultrasound-guided fine needle aspiration (EUS-FNA) biopsy is increasingly used in the diagnosis of pancreatic lesions. Although EUS-FNA has a high specificity in diagnosing malignancy, the reported sensitivity is variable. FNA diagnosis can be achieved by cytomorphologic evaluation alone but ancillary studies may be needed in some cases. Rapid on-site evaluation helps ensure adequate sampling appropriate specimen triage, thus enhancing diagnostic performance. In this study, we retrospectively reviewed our experience in diagnosing solid pancreatic lesions via EUS-FNA biopsy.

Design: The electronic database of cytopathology was searched for pancreatic lesions diagnosed by EUS-FNA during the period from January 2005 to June 2011. We identified 1,143 cases, of which 681 were solid lesions. The final cytology diagnoses included non-diagnostic, negative, indeterminate (atypical and suspicious), neoplasm, and malignant (primary or metastatic). Most cases had rapid on-site evaluation performed. Histopathologic follow-up was available for comparison in 151 cases (22%).

Results: Of 681 cases, 23 cases (3%) were non-diagnostic. Negative, indeterminate, neoplastic, and malignant diagnoses were rendered in 115 (17%), 59 (9%), 58 (9%), and 426 (62%), respectively. Cytologic diagnoses and histopathologic follow-ups were compared in 151 cases (see Table 1). The overall concordance rate for negative, neoplastic and malignant diagnoses was 92%. One of 93 cases that were cytologically diagnosed as malignant showed autoimmune pancreatitis in the follow-up. Neoplastic or malignant diagnosis was seen in 6 of 11 cases that had a negative cytological diagnosis. The calculated sensitivity, specificity, positive predictive value, and negative predictive value were 95%, 83%, 99%, and 45%.

Table 1. Correlation between Cytologic Diagnosis and Histopathologic Follow-up

	Cases	Histopathologic Follow-up		
Cytologic Diagnosis	n	Negative	Benign Neoplasm	Malignant
Non-diagostic	2	2 (100%)	0	0
Negative	11	5 (46%)	2 (18%)	4 (36%)
Indeterminate	23	3 (13%)	2 (9%)	18 (78%)
Neoplasm	22	0	19 (86%)	3 (14%)
Malignant	93	1 (1%)	0	92 (99%)
Total	151	11 (7%)	23 (15%)	117 (78%)

Conclusions: In this one of the largest series, we demonstrate that solid pancreatic lesions can be accurately diagnosed by EUS-FNA with 3% non-diagnostic and 9% indeterminate. EUS-FNA has high sensitivity and high positive predictive value in diagnosing pancreatic malignancy. Adequate sampling and awareness of diagnostic pitfalls may help avoid false positive and false negative diagnoses.

344 Radiologic and Clinical Predictors of Malignancy in the Follicular Lesion of Undetermined Significance

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Background: The Bethesda 2007 Thyroid Cytology Classification defines follicular lesion of undetermined significance (FLUS) as a heterogeneous category of cases that are not convincingly benign nor sufficiently atypical for a diagnosis of malignancy. Various ultrasonographic characteristics of a thyroid nodule have been associated with a higher likelihood of malignancy, and certain clinical features may also increase the likelihood of malignancy in patients. This study is designed to determine the ultrasonographic and clinical predictors of malignancy in the FLUS category.

Design: A search through the cytology files at our institution from January 2008 to December 2010 was made for cases with diagnosis of FLUS. Cases with follow-up surgical intervention formed the cohort of this study. Surgical pathology and ultrasonographic findings were reviewed. Clinical information was obtained from medical records. The clinical and radiologic findings were correlated with the final surgical pathology diagnosis.

Results: A total of 140 cases of FLUS with corresponding surgical intervention were identified (112 females and 28 males). There was 75% malignancy rate in nodules with irregular contours, compared with 50% in nodules with regular outlines. Nodules demonstrating calcifications showed 57% malignancy rate, compared with 50% in nodules without calcifications. Sixty one percent of cases with a final ultrasound diagnosis of indeterminate to suspicious were positive on surgical resection. Malignancy rate in solid nodules was 50%. The rates of malignancy in patients with radiation exposure, symptomatic nodules and positive family history of thyroid cancer were 22%, 57% and 33%, respectively. BRAF Mutation was demonstrated in 57% of malignant cases and none of benign cases.

Conclusions: No single clinical or ultrasonographic feature or combination of features is adequately sensitive or specific to identify all malignant nodules. However a combination of solid nodules, nodules with irregular contours, symptomatic nodules and positive BRAF mutation have high predictive value for malignancy in patients with cytologic diagnosis of FLUS.

345 Utility of BRAF Gene Testing on Thyroid Nodules Diagnosed as Follicular Lesion of Undetermined Significance (FLUS)

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Background: The evaluation of thyroid nodules routinely begins with fine needle aspiration (FNA) to triage patient management based on the risk of malignancy. The Bethesda System for Reporting Thyroid Cytopathology (TBSRTC) has a category for borderline cases of follicular lesions that do not satisfy all criteria for malignancy. At our institution, we have a 3% rate of diagnosing a lesion as FLUS, based on a computerized search of 2972 cases from 2003-2007. Previous studies have shown that BRAF mutations variably occur in 46-69% of papillary thyroid carcinoma (PTC). Within certain histological subtypes of follicular variant PTC, 26% of cases have also been shown to contain the BRAF mutation. This study assess the value of BRAF mutation analysis by PCR in FNA specimens previously labeled as FLUS.

Design: From March 2007 to March 2011, 36 cases at our institution were diagnosed as FLUS and later had a surgical pathology tissue diagnosis. We used 8 benign and 11 malignant cases of FLUS that appeared to have adequate cells. Genomic DNA was extracted from cells on the previously stained cytology slides, and BRAF V600E mutation analysis was performed by a real-time PCR approach utilizing BRAF wild-type and V600E Taqman probes.

Results: Of the 19 cases selected based on cytology slides, 15 had sufficient DNA for mutation analysis. BRAF V600E mutation was detected in one case (6.7%), which was confirmed as PTC on surgical excision. None of the follicular variant PTC cases tested positive for BRAF mutation. When considering cases with sufficient DNA, the sensitivity of BRAF mutation to detect PTC is 11% and the negative predictive value is 43%. The positive predictive value and specificity are 100%.

BRAF Mutation Compared to Resection Diagnosis

	Tissue Diagnosis		
BRAF Mutation	PTC, any variant	Benign	TOTAL
Detected	1	0	1
Negative	8	6	14
TOTAL	9	6	15

Conclusions: We show a lower sensitivity and negative predictive value for BRAF mutation testing in FLUS thyroid nodules, compared to previous studies. These differences may be attributed to a smaller sample size. The specificity for BRAF testing is high, and BRAF testing can be a useful adjunct test. Although BRAF mutation analysis by a real-time PCR approach on previously stained cytology slides is technically possible, not all cytology slides will have adequate cells for DNA extraction after repeated processing. Given its high specificity, it may be worthwhile to obtain tissue dedicated to BRAF testing at the time of FNA.

346 Diagnosis of Upper Tract Urothelial Carcinoma by Urinary Cytology: Evaluation of Its Efficacy and Limitations

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Background: Primary upper urinary tract (UT) urothelial carcinoma (UC) is rare. Upper tract washing cytology is often used during UT surveillance. The diagnosis of UTUC with urinary cytology is often challenging. Reports regarding the efficacy of UT cytology are limited. We herein study the value of UT cytology in detecting UTUC in a large cohort.

Design: One hundred UT cytologic specimens were retrieved from our database during a ten-year period (2001-2011). For each patient, the cytology specimen with the highest degree of abnormality was selected. The histologic sections of these cases were also studied.

Results: Sixty-seven washings were obtained from ureter and 33 were from renal pelvis. 76 cases had histologic follow-up either by serial ureterorenoscopic biopsies or nephroureterectomy. Among them, the cytologic diagnosis of positive or suspicious for high-grade UC was made in 15 cases; suspicious for low-grade UC in 3 cases; atypical urothelial cells in 19 cases; and negative in 39 cases. Of the 15 washings with diagnosis of positive or suspicious for high-grade UC, 10 had histologically confirmed high-grade UC, 1 had low-grade UC on histology, and 4 had benign histology. All 3 cases of cytologically suspicious for low-grade UC had low grade UC on concomitant histology. Among the 19 washings with atypical urothelial cells, 7 were low-grade UC, 1 was high-grade UC; and 11 were benign on histology. Six of 39 cases with negative cytology had UC (3 low-grade and 3 high-grade) on histology. Combining positive and suspicious for UC diagnoses, sensitivity and specificity for detecting high-grade UC were 71.4% and 91.9%, while for low-grade UC were 21.4% and 100%, respectively.

Conclusions: UT washing cytology has high specificity for detecting high-grade UC. However, sensitivity for detecting UC is low, especially for low-grade UC. Practical value of UT washing cytology needs to be further evaluated in future studies.

347 Endoscopic Ultrasound (EUS)-Guided Fine Needle Aspiration of Metastatic Malignancies Involving the Pancreas: A Single-Institution 10-Year Retrospective Study of 46 Cases

S Chen, X Wang, H Cramer. Indiana University, Indianapolis.

Background: Metastatic malignancies involving the pancreas are relatively uncommon compared to primary pancreatic neoplasms. Documentation of metastases to the pancreas by fine needle aspiration (FNA) sometimes obviates the need for surgery entirely and allows the oncologist to institute the appropriate course of medical treatment.

Design: A computerized search of our laboratory information system was performed for the 10-year period from April 2001 through March 2011 to identify all metastatic neoplasms involving the pancreas diagnosed by FNA. All correlating surgical pathology reports were also reviewed and the microscopic slides from selected cases were re-examined.

Results: Over the 10-year period encompassed by this study, a total of 4369 FNAs of the pancreas were performed at our institution which included 1445 cases of pancreatic ductal adenocarcinomas (33%) and only 46 cases of metastatic malignancies involving the pancreas (1%). The male to female ratio was 1:1.7. The metastatic lesions ranged in size from 1.2 to 6.0 cm with a mean of 3.2 cm. There were 13 cases diagnosed by FNA as metastatic renal cell carcinoma (RCC), 2 cases diagnosed as suspicious for metastatic RCC and 2 cases of primary pancreatic adenocarcinoma that were misclassified as metastatic RCC. One of the misclassified cases had a prior history of RCC while the other patient presented with synchronous pancreatic and renal masses. There were 12 cases of metastatic small cell carcinoma originating from a lung primary, 7 cases of metastatic adenocarcinomas originating from breast (3), colon (3) and esophagus (1) and 4 cases of metastatic melanoma originating from primaries in the arm (2), gallbladder (1) and unknown site (1). The remaining 6 metastatic malignancies included neuroendocrine carcinoma from a head and neck primary (2), urothelial carcinoma arising from a renal primary (1), squamous carcinoma from the anus (1), low grade sarcoma from the thigh (1) and a leiomyosarcoma from the abdomen (1). Immunocytochemical staining was performed in only 4 of these 46 cases, and histopathologic follow-up was obtained in only 10 cases.

Conclusions: Metastatic lesions to the pancreas are relatively uncommon. The most common metastatic tumors to the pancreas are RCC, small cell carcinoma of pulmonary origin, adenocarcinoma and melanoma. The 2 diagnostic errors (4%) suggest that clinical data can sometimes adversely influence cytomorphologic interpretation and that perhaps more frequent utilization of appropriate immunostains could conceivably reduce our error rate.

348 Fine Needle Aspiration of the Mediastinal Lesions: A 20-Year Retrospective Study of 561 Cases

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Background: Image-guided fine needle aspiration (FNA) has been used routinely for diagnosing mediastinal lesions. In this study, we retrospectively reviewed our 20-year experience with FNA of the mediastinum and determined its diagnostic accuracy and clinical utility.

Design: A computerized search of our cytology database was performed for all FNAs of the mediastinum from 1990 to 2010. All cytology reports and correlating surgical reports were reviewed and slides from selected cases were re-examined.

Results: A total 561 FNA cases of the mediastinum were performed over a 20-year period. There were 329 malignant cases (59%), 157 benign cases (28%), 22 cases suspicious for neoplasm/atypical cells (4%), and 53 unsatisfactory cases (9%). Malignant cases included metastatic carcinoma (169 cases, 51%), thymoma/thymic carcinoma (45 cases, 14%), germ cell tumor (43 cases, 13%), lymphoma/leukemia (34 cases, 10%), primary neuroendocrine tumor (10 cases, 3%), and other rare poorly differentiated tumors (28 cases, 9%). Histological correlation was available for 238 FNA cases (42%) including 148 malignant, 57 benign, 11 suspicious for neoplasm/atypical cells, and 22 unsatisfactory cases. There were 32 patients diagnosed as metastatic small cell carcinoma by FNA who were directly referred for chemotherapy without ever obtaining a confirmatory surgical biopsy. Among 148 malignant cases, 141 (95%) cases were confirmed histologically; 4 malignant cases (3%) lacked histological confirmation due to biopsy sampling error and 3 cases (2%) were incorrectly classified by FNA including 1 case of lymphangiomyomatosis misdiagnosed as teratoma, 1 case of small cell carcinoma misdiagnosed as large cell lymphoma, and 1 case of sclerosing large cell lymphoma misdiagnosed as thymoma. Among 57 benign cases, 40 cases (70%) were confirmed histologically. However, there were 14 false negative FNA cases (25%) including 5 Hodgkin lymphomas, 3 adenocarcinomas, 2 large B cell lymphomas, 1 low grade neoplasm, 1 small cell carcinoma, 1 malignant peripheral nerve sheath tumor and 1 thymic carcinoma. Among 11 cases diagnosed as suspicious for neoplasm/atypical cells, 10 were proven to be neoplasms by follow-up histologically.

Conclusions: In this study, the overall diagnostic accuracy for the FNA diagnosis of mediastinal lesions was 88% and the misclassification rate was low (2%). False negative and unsatisfactory diagnoses were due primarily to sampling error. In our experience, FNA is a valuable method for the diagnosis of mediastinal lesions and is particularly beneficial for the management of patients with small cell carcinoma.

349 MicroRNA Expression in Lymph Node Fine Needle Aspiration Biopsy

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Background: microRNAs (miRs) are small noncoding RNAs with regulatory roles in the fine tuning of protein expression at the level of posttranscriptional regulation. Since their discovery, miRs have been identified as playing major roles in the regulation of numerous normal biologic processes. Their deregulation was linked to multiple pathologic processes (inflammation and especially cancer). Chronic lymphocytic leukemia (CLL) is one of the most frequent adult leukemias in the Western world. Recently, Croce et al identified the deletion of miR15 as being present in 65% of analyzed CLL cases. Further investigation revealed that so-called "indolent" CLL is most likely to be associated with the deletion of miR15.

Design: We investigated whether miR expression in cells collected using the technique of fine needle aspiration (FNA) could identify a specific genetic signature for different leukemias/lymphomas. FNA was performed on lymph nodes of 15 patients: 8 reactive (lymphoid hyperplasia or inflammation), 1 monoclonal T cell population proliferation, 1 diffuse large B cell lymphoma, 1 follicular lymphoma and 4 CLL. Flow cytometry confirmed the immunophenotype of these lesions. RNA was extracted from all samples with Trizol (Invitrogen, Carlsbad, California) and real time PCR was performed with Taqman probes (Applied Biosystems) for miR146a, miR29a, miR155, miR15 and miR16 - all miRs known to be involved in the pathogenesis of various leukemias/lymphomas, especially CLL. The comparative C_T (threshold cycle) method for relative quantitation of gene expression was used to determine miR expression levels.

Results: Extraction of RNA and identification of specific miR expression was possible and adequate in all cases. Of all miRs analyzed, miR15 seemed to best predict the presence of a CLL with an indolent course. Both such CLL samples had a very low level of miR15 amplification, whereas all other samples (including 2 aggressive CLLs) exhibited higher miR15 levels, ranging from twice to > 20 times higher.

Conclusions: MiR expression using FNA samples in non-Hodgkin lymphomas/leukemias is technically possible and can provide important clues not only to possible subtype but also to clinical course. Based on our preliminary results we hypothesize that miR expression can be used together with more classical ancillary techniques (flow cytometry, immunohistochemistry, FISH) to identify and predict the behavior of CLL and possibly other non-Hodgkin lymphomas. It may eventually also be used to monitor a patient's response to treatment.

350 Assessment of On-Site Evaluation (OSE) and Diagnostic Yield of EUS-Fine Needle Aspiration (FNA) Versus EUS-Guided Needle Core Biopsy Using EchoTip ProCore (ETP) Device

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Background: EUS is an established procedure for diagnosing and staging gastrointestinal (GI) lesions. Usually FNA is used to obtain lesional material, but can have low yield and sensitivity for certain tumors or anatomic locations limiting material available for diagnosis. Our pilot study used a recently introduced ETP device (Cook Medical, USA) and showed it to be technically feasible and have a high diagnostic yield. Here we assessed OSE and diagnostic yield of ETP compared to FNA.

Design: 46 cases that had tandem FNAs and ETPs were evaluated. Between 3-8 FNA and 1-5 ETP passes were performed based on OSE. For all cases, FNA smears, FNA cell blocks (CB), touch preps (TP) of ETPs, and ETP slides were reviewed. Diagnostic rates of both techniques were evaluated. IHC was performed when appropriate.

Results: 38 cases with definitive diagnoses were evaluated. 6 "unsatisfactory" and 2 "atypical" diagnoses were excluded. Final diagnoses were possible by FNA/CB in 26 cases. Diagnoses could be rendered by ETP/TP in 33 cases.

There were 14 adenocarcinomas (AC) and 24 other lesions. Amongst our AC, a diagnosis was reached by FNA/CB in 12 cases and ETP/TP in all 14 cases. Subjectively, we found both techniques to be equivalent in terms of diagnostic material provided.

Amongst the 24 non-AC (1 squamous cell carcinoma, 2 lymphomas, 12 spindle cell lesions, 4 endocrine tumors, 4 inflammatory lesions, 1 benign), definitive diagnoses could be reached by FNA/CB in 14 cases, and by ETP/TP in 19 cases. Amongst our non-ACs, the most common tumors were GISTs. FNA/CB and ETP/TP were each diagnostic in 5 of 7 GISTs.

As far as OSE, specimens that were ultimately diagnostic on ETP had adequate TP OSE 60% of the time, whereas diagnostic FNA/CB specimens had adequate OSE 61% of the time. Adequacy assessments were particularly unreliable in GISTs (adequate in diagnostic cases less than half the time by either technique).

Conclusions: TP/ETP provided diagnostic material more frequently than FNA/CB in both ACs and non-ACs. The predictive rate of OSE could possibly be improved by performing crush preps rather than TPs on portions of the ETP, particularly in cases of suspected spindle cell lesions. In cases that were ultimately diagnostic, ETP provided adequate material within 4 passes 85% of the time, suggesting its utility as a stand-alone technique in settings where OSE is not available.

351 The Value of Mutational Profiling of the Cytocentrifugation Supernatant Fluid from Fine Needle Aspiration of Pancreatic Solid Mass Lesions

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Background: Fine needle aspiration (FNA) of pancreatic solid masses can be significantly impacted by sampling variation. Molecular analysis of slide based atypical cells has been reported as an aid for a more definitive diagnosis. The aim of this study is to evaluate how the molecular analysis of the cell-free DNA can help reducing sampling variability.

Design: FNA smears from 19 pancreatic solid masses were made. The remaining aspirate was diluted for preparation of cytocentrifuged slides or cell blocks. The supernatant fluid underwent DNA extraction and mutational analysis. DNA was extracted from 2 ml of the cytocentrifugation supernatant fluid and the DNA quantity measured by optical density. qPCR was used to determine DNA amplifiability. Aliquots of the extracted DNA underwent mutational analysis using PCR/capillary electrophoresis for a broad panel of markers (KRAS point mutation by sequencing, microsatellite fragment analysis for loss of heterogeneity of 16 markers at 19, 3p, 5q, 9p, 10q, 17p, 17q, 21q, 22q). In selected cases, microdissection of stained cytology smears and/or cytocentrifugation cellular slides were similarly analyzed and compared.

Results: 5/19 cases cytologically confirmed as malignant showed detectable mutational change both in the microdissected slide based cytology cells and the cytocentrifugation supernatant fluid. While most mutations detected were present in both microdissected slides and supernatant fluid specimens, the latter showed additional mutations supporting greater sensitivity of detection. Mutations present in both types of specimens were concordant affecting the same parental allele or KRAS point mutation genotype. The proportion of DNA mutated for individual markers (clonality) was higher in the supernatant fluid than in microdissected cells.

Conclusions: 1. The cytocentrifugation supernatant fluid contains adequate levels of amplifiable DNA suitable for mutation detection and characterization. 2. The finding of additional detectable mutations at higher clonality indicates that the supernatant fluid may be enriched with tumor DNA. 3. The molecular analysis of the supernatant fluid could serve as an adjunct method to reduce sampling variability, especially in cases with a high clinical suspicion for malignancy and limited number of atypical cells in the smears.

352 Lung Sarcomatoid Carcinoma (SC): EGFR Mutation Analysis on Fine Needle Aspiration Biopsy (FNAB) with Clinicopathologycal Study (23 Cases)

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Background: SC is a rare group of non small-cell lung carcinomas (NSCLC) with sarcomatous differentiation. Five subgroups are recognized (pleomorphic, spindle cell, giant cell, carcinosarcoma and pulmonary blastoma). Tobacco is the major etiologic

factor implicated. Asbestos exposure may also be related. Prognosis is worse than others NSCLC. Clinical outcome is stage dependent with poor response to conventional treatment.

Design: The aim is to determine the EGFR mutations on cytology samples from SC and correlate the results with clinical stage, treatment and outcome. 23 cytology samples diagnosed as SC were collected from our files (2001 to 2011): 2 sputum and 21 FNAB (16 lung primary lesions and 5 metastasis -2 bones, 1 chest wall, 1 adenopathy and 1 skin). Cytologyc diagnosis was confirmed by histology (9 surgical specimens, 10 cell block and 1 autopsy). DNA was extracted and EGFR mutation was detected by polymerase chain reaction followed by restriction enzyme digestion and sequencing of exon 19, 20 and 21. Follow up, treatment and outcome were evaluated from clinical reports.

Results: 20 cases were men and 3 women. The median age was 62y (39-96). 17 were smokers, 2 non-smokers (1 exposed to asbestos) and 4 unknown. In 85 % the lesion was located in the right pulmonary lobe. The up/down lobe ratio was 3/1. Average tumor size was 5.3cm (1-12.4). The clinical stage at the diagnosis was IV (47.8%), IIIB (4.34%), IIIA (17.39%), IIB (8.69%), IB (4.34%), unknown (17.39%). Stages IB, IIB and IIIA were treated with surgery, chemotherapy (CT) and some with combined radiotherapy (RT). Stages IIIB and IV were treated only with CT and RT. 14 patients were dead (stage IV in 57.1%) with a mean survival of 13.7 months, 7 are alive with a mean survival of 22.2 months and 2 with unknown outcome. The EGFR results were: 1 not available, 20 wild-type and 2 cases mutated on exon 21 (L858R) which were in stage IV. The treatment they received didn't change the outcome. They die after short survival.

Conclusions: Our results are similar as those from other series. SC has a poor prognosis. The diagnosis is made in advanced stage. The tumor is more frequent in men and in the upper lobes. EGFR mutation in our series is 9.1%, all of them in stage IV. Further studies are needed to conclude if EGFR inhibitors could improve the survival rates in patients with EGFR mutation.

353 ER, PR, HER-2/Neu Immunostaining in Cytology: Effect of Varied Fixation on Human Breast Cancer Cell Lines

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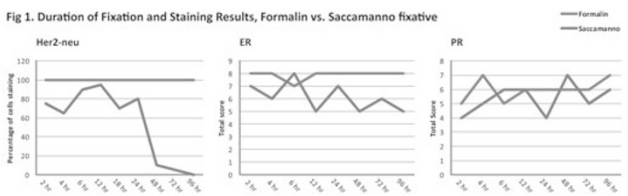
Background: The ASCO/CAP Expert Panel recommends that all invasive breast carcinomas and breast cancer recurrences be tested for ER, PR and HER-2/neu expression. The guidelines for the testing of surgical specimens by immunohistochemistry (IHC) are well defined, whereas they are lacking for cytological samples. This study evaluates various fixation protocols for optimal receptor testing by IHC of human breast cancer cell lines.

Design: Human breast cancer cell lines MCF-7 (ER/PR positive) and SKBR-3 (overexpressing HER-2/neu) were used. The cells were fixed in 10% neutral buffered formalin and Saccomanno Fixative (SF) for 2, 4, 6, 12, 24, 48, 72 and 96 hours, and embedded in paraffin as cell blocks. IHC was performed on a Dako autostainer using primary monoclonal antibodies for ER (clone 1D5, Dako), PR (clone PGR 636, Dako) and HER-2/neu (Zymed). ER and PR slides were assigned a proportion score (PS; 0-5), an intensity score (IS; 0-3) and a total score (TS=PS+IS). For the evaluation of HER-2/neu staining we used the standard DAKO scoring system ranging from 0 to 3+.

Results: When stained for ER, formalin-fixed cells had TS of 8 for majority time points, while SF-fixed cells had TS ranging from 8 at 6h to 5 at 96h. TS for PR were more variable for both fixatives, varying between 5 and 7 with formalin fixation and between 4 and 7 with SF. HER-2/neu staining for the formalin-fixed cells was uniformly 3+ for all time points. The SF-fixed cells showed patchy 2+ to 3+ staining when fixed up to 24h; however, if cells were fixed more then 24h, the scores were 0-1+.



Conclusions: Human breast cancer cells can be successfully stained for ER, PR and HER-2/neu when fixed in formalin. In contrast to 6-72h fixation recommended for ER/PR staining and 6-48h fixation for HER-2/neu staining for surgical specimens, the fixation time for formalin-fixed cells may be broader - ranging from 2h to 96h. Cells fixed in SF from 2 to 96 hours will also stain well for ER and PR. SF produces variable results for HER-2/neu staining, in particular, SF fixation beyond 24h may cause false negative results.

354 Comparison of HR HPV Positive Rates Using the HC2 Versus the Cervista Test in Women 30 Years of Age or Older with NILM Cytology Results and Clinical Follow-Up

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Background: The FDA approved Cervista HPV HR test similarly to the Hybrid Capture 2 (HC2), is used as a triage test for women with ASC-US cytology and as an adjunctive screening test in women 30 years or older. Advantages of the Cervista compared to the HC2 are smaller sample requirement and an internal DNA control. Studies showed that HPV HR positivity rates in women 30 or older with NILM cytology results are similar with the two methods. While there was concern about the performance of Cervista in the older women, studies have shown similar HPV HR positive rates as age increases with both tests. The goal of study was to compare HPV HR positive rates with HC2 and Cervista in women 30 or older with NILM cytology results.

Design: We retrospectively reviewed cervical cytology and HPV test results using the HC2 method from March 2008 to February 2009 from women 30 years of age or older with NILM cytology and positive HPV results. Similar selection criteria were used for the period of May 2009 to April 2010, with the difference that HPV testing was done by the Cervista test. Clinicopathologic follow-up data was reviewed in both groups.

Results: For the HC2 group 2311 women met our criteria. There were 138/2311 (5.97%) women with NILM Pap and HPV HR positive results. In the Cervista group 2213 women were included, and 135/2213 (6.1%) had NILM cytology and positive HPV HR results. Clinical follow-up was available in 23 (17%) of patients from the HC2 and 22 (16.2%) from the Cervista group.

Follow-up in the HC2 group (23): NILM, HPV – (11); NILM, HPV+ (4); ASC-US, HPV+ (4); CIN II (2); CIN III (1).

Follow-up in the Cervista group (22): NILM, HPV – (15); NILM, HPV+ (3); ASC-US, HPV+ (1); CIN I (1), CIN II (1); CIN III (1).

Conclusions: HR HPV positivity rates in women 30 or older with NILM cytology are comparable with two methods (5.97% vs. 6.1%).

While follow-up data was available for only a small number of patients, majority had negative (NILM, HPV-) follow up results, regardless of which HPV test was used.

Importantly both tests identified a small subset of women with clinically significant lesions, reflecting comparable sensitivity of the two tests.

Per ASCCP guidelines women 30 or older with NILM cytology and positive HR HPV test results should undergo repeat testing (Pap and HPV) at 12 months. Our data suggest that these recommendations are not strictly followed. While some women undergo repeat testing or further work-up sooner than 12 months, majority of patients had no follow-up.

355 Hybrid Capture 2 Test Results after an Initial Equivocal RLU/CO Value Are Dependent on Age

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Background: Manufacturer recommended interpretation guidelines for the high risk human papilloma virus (HPV) test Hybrid Capture 2 (HC2) require retesting specimens with an initial equivocal result, defined as a Relative Light Unit/ Cut Off (RLU/CO) value between 1 and <2.5. While a retest with RLU/CO≥1 leads to a "positive" HC2 result, a lower value requires a second retest which is then considered final ("positive" if RLU/CO≥1). However, we have previously documented a low predictive value for cervical intraepithelial neoplasia 2 or worse in women ≥50 years with specimens within the equivocal range and questioned the use of a single interpretative algorithm regardless of age. Therefore, we now decided to investigate the influence of age on HC2 final results produced by the current interpretative algorithm for initial equivocal RLU/CO values.

Design: We identified 365 consecutive cervico-vaginal liquid cytology specimens with initial HC2 HPV tests within the equivocal range. Retest and second retest RLU/CO values were compared for women of three different age groups (15-29, 30-49 and ≥50 years).

Results: The proportion of retests with RLU/CO<1 increased with age (p<0.001 based on Chi-square test).

Retests By Age Group

Age in Years	RLU/CO<1	RLU/CO≥1	Total
15-29	12 (7.4%)	151 (92.6%)	163
30-49	28 (20.1%)	111 (79.9%)	139
≥50	17 (27%)	46 (73%)	63
Total	57	308	365

59 second retests were performed out of which only 7 had RLU/CO≥1. The proportion of second retests with RLU/CO<1 vs RLU/CO≥1 was not statistically significant (p=0.140). Overall, the proportion of "positive" HC2 results following the current interpretative algorithm decreased with increasing age (p<0.001).

Conclusions: HC2 test results after an initial equivocal RLU/CO value are clearly dependent on age. The likelihood of a retest with RLU/CO≥1 resulting in a "positive" HC2 result decreases significantly with increasing age. These laboratory results appropriately parallel the known HPV infection prevalence in the general population.

356 Trend of Population Coverage, Frequency and Volume of Pap Tests: An Attempt To Estimate the Extent of Unnecessary Pap Tests in the Era of HPV-Testing

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Background: Screening for cervical cancer has changed remarkably. We and others have predicted a decrease in Pap test volume regardless of demographic change. In this report, we assessed those predictions by: 1) determining the changes in population coverage and trends in the frequency and volume of Pap tests 2) estimating volume of unnecessary Pap tests and 3) assessing the correlates associated with unnecessary Pap tests.

Design: This is a secondary data analysis of the NHIS for 2000, 2005, 2008 and 2010. It focused on questions related to ever having a Pap test, number of Pap tests in 6 years, having an abnormal Pap test, hysterectomy status, socio-demographic factors, health status and extent of access to health care. We used the Census Bureau to estimate the population of women eligible for screening. After multiple imputations, we used logistic regression SAS MI-analysis to determine the correlates associated with unnecessary Pap tests.

Results: Beween 2000 and 2010, the population of women eligible for screening increased by 12%, 108 to 121 million,while the national Pap test volume remained the same, 66 .4 million, 95% C.I. 64.7-67.9 million, vs. 66.0 million, 95% C.I. 63.9-66.2 million. Coverage with Pap test in the preceding year was significantly higher in 2000 at 66%, 95% C.I. 65.1-66.6%, compared to 2010, 55.5%, 95% C.I. 54.4-56.6%, while coverage in the preceding 3 years remained the same, 84% in 2000 compared to 79.4% in 2010. Within the group of women age >20 who had normal Pap tests, a significantly

higher percent received an annual Pap test in 2000, 56.1%, C.I. 54.9-57.3%, compared to 2010, 52.8%, 95% C.I. 51.5-54.2%. The volume of unnecessary Pap tests was not different in 2000, 21.0 million, 95% C.I. 20.3-21.7 million, compared to 22.4 million, 95% C.I. 21.5-23.4 million, in 2010. A Factors that were significantly associated with having unnecessary Pap tests included having health insurance coverage, odd ratio (OR) 1.5, 95% C.I. 1.2-1.9, number of mammograms, OR 3.7,95% C.I. 3.1-4.3, having visited an ob/gyn clinic, OR 3.9, 95% C.I. 3.4-4.7, having a doctor recommending Pap test, OR 1.2,95%, C.I. 1.01-1.3 and being married, OR 1.36, 95% C.I. 1.1-1.7.

Conclusions: Although Pap test volume has decreased compared to demographic changes, still one third of this volume is performed unnecessarily, in women who have frequent and easy access to healthcare. Physician and patient education is needed to reduce unnecessary Pap tests.

357 FNA Is a Highly Accurate Procedure for Detecting Axillary Lymph Node Metastases in Breast Cancer Patients

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Background: Patients with biopsy proven breast cancer often undergo lymph node staging prior to surgery or chemotherapy. Recently, at our institution there was a switch from fine needle aspiration (FNA) to predominately core needle biopsy for evaluating atypical axillary lymph nodes, as the radiologists believed more tissue for examination would yield better diagnostic results. While a comparison of these two modalities is the ultimate goal, this study initially sought to determine the diagnostic accuracy of axillary lymph node FNA in patients diagnosed with breast cancer.

Design: A computer search identified patients who underwent axillary lymph node FNA after diagnosis of breast cancer. The pre-test suspicion by ultrasound criteria was noted. The size and histology of the breast tumor, the number of FNA passes performed, and histologic follow-up of the lymph nodes were recorded. For neoadjuvant cases or those without surgical follow-up, two independent cytologists blinded to the cases re-reviewed the FNA cytology. The study was approved by the University of Utah IRB.

Results: 39 breast cancer patients (women, 30 to 89 years of age) underwent axillary FNA from 2003-2011. Tumors were predominantly ductal (97%). From 2003-2009, a cytopathologist performed FNA's on palpable nodes. After 2009, most were performed by a radiologist with an experienced cytopathologist on site for immediate assessment. 14 FNA's were palpable, and 25 FNA's were performed by ultrasound-guidance. 1 (2.6%) case was non-diagnostic but was followed by core biopsy. 2 cases were excluded from analysis. FNA performed by either palpation or ultrasound showed similar results. Overall, 10 (27%) FNA's were called negative and 27 (73%) were called positive or atypical. The sensitivity of FNA was 96% and the specificity was 90%.

Conclusions: FNA is a simple and highly accurate technique for diagnosing axillary lymph node metastases in suspicious lymph nodes. Immediate assessment ensures proper management at the time of the procedure. Additional tissue obtained by core biopsy is not necessary for diagnosis.

358 The Combination of HBME-1 and Galectin-3 with BRAF-1 Mutation on Liquid-Based Cytology Identifies High-Risk Follicular Thyroid Lesions

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Background: Thyroid nodules are common findings in adults, but only few of them are malignant. Fine needle aspiration cytology (FNAC) is the most important tool for evaluating these lesions which may yield an indeterminate diagnosis (Follicular Neoplasm – FN). To decrease the FN diagnoses, mutations of BRAF gene and immunocytochemistry (ICC) for HBME-1 and Galectin-3 have been studied. BRAF-1 mutations involving the V600E locus have been identified in as much as 70% of classic and tall cell (TCV) variants of PC and are regarded as useful in improving the specificity of the FNAC. They have not been detected in benign lesions and in the majority (80%) of the follicular variants of PTC. This study focuses on the combination of an ICC panel (HBME-1 and Galectin-3) with BRAF mutation for refining the indeterminate cytologic (including atypical cells of undetermined significance – AUS – and FN) and suspicious for carcinoma (SC) diagnoses.

Design: Among 34 FNAB processed by LBC, 9 were FN (including AUS) and 25 were SC. The ICC expression of HBME-1 and Galectin-3 and the BRAF V600E mutation were investigated with the ThinPrep 2000 (Hologic Co, Marlborough MA) technique. Immunocytochemical staining were carried out with the avidin-biotin peroxidase complex on LBC slides. The DNA extraction for the mutational analysis was performed on the same LBC material using the QIAamp tissue kit (Qiagen, Hilden, Germany) with the PCR amplification of the exon 11.

Results: Seven FN/AUS and 15 SC underwent surgery. In the surgical series 7 positive BRAF cases and 9 ICC positive cases were found. The results are shown in the Tables.

Table 1: BRAF-1 mutation analysis on 22 surgical patients

SURGICAL SERIES	FOLLICULAR NEOPL. (INCL. AUS)		SUSPICIOUS FOR CARCINOMA	
	BRAF+	BRAF-	BRAF+	BRAF-
BENIGN	0	3	0	8
PTC/TCV	0	0	6	0
FVPTC	0	4	1	0

PTC/TCV (papillary thyroid carcinoma/tall cell variant) FVPTC (follicular variant of papillary carcinoma)

Table 2: Immunocytochemical expression of HBME-1 and Galectin-3 on 16 surgical cases

SURGICAL SERIES	FOLLICULAR NEOPL. (INCL. AUS)		SUSPICIOUS FOR CARCINOMA	
	ICC +	ICC -	ICC +	ICC -
BENIGN	0	3	1	1
PTC/TCV	0	0	6	0
FVPTC	1	3	1	0

Conclusions: BRAF-1 mutations and expression of HBME-1 and Galectin-3 on LBC enhance the accuracy of FNA in detecting thyroid malignancy. ICC seems to be more effective in identifying malignancies in lesions with indeterminate cytology (FN/AUS) whereas BRAF-1 mutation analysis discovers more carcinomas in suspicious lesions.

359 Endoscopic Ultrasound-Guided Fine Needle Aspiration of Pancreatic Neuroendocrine Tumors: Is Accurate Grading Based on the 2010 ENTS/WHO Criteria Possible on Cytologic Specimens?

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Background: The natural history of pancreatic neuroendocrine tumors (panNETs) is highly variable, and one of the most controversial aspects in the diagnosis of these tumors is prediction of their clinical behavior. Some panNETs that behave in a malignant fashion may have deceptively bland cytologic features and, conversely, some with previously described "malignant" criteria may not always prove lethal. Various classification schemes have been proposed for panNETs, the most recent of which includes assessment of mitotic count and/or Ki-67 labeling index (WHO 2010 and European Neuroendocrine Tumor Society proposals). We undertook this study to determine if FNA cytology samples can be accurately graded comparable to histologic material based on the 2010 ENTS/WHO criteria.

Design: The archival files were retrospectively searched for panNET cases diagnosed on endoscopic-ultrasound guided fine needle aspiration (EUS-FNA). Those cases with adequate cell block and correlating histological material were retrieved for inclusion in the study. Paraffin-embedded formalin-fixed cell blocks and representative tissue blocks from the surgical specimens were immunostained with Ki-67 using standard techniques. The cytological samples were assessed for positive staining in tumor cells, and the proliferative index was calculated by dividing the number of positive tumor cells by the total number of tumor cells present in the cell block. The correlating histological material was graded in a blinded fashion using the ENTS guidelines, and the results compared to those derived from the cytologic evaluation.

Results: Ten out of 11 cases (91%) had equivalent grading scores when comparing Ki-67 proliferation rates in cell block material to histological material. Of these, 5 of 11 cases were interpreted as grade 1 (Ki-67 index ≤2%), and another 5 of 11 cases were interpreted as grade 2 (Ki-67 index >2-20%). One case was interpreted as grade 2 on cell block material and grade 1 on histological evaluation.

Conclusions: Although larger studies with inclusion of high grade tumors are needed for more accurate validation, the results of this study indicate there is reliable correlation of tumor grade based on ENTS criteria when comparing EUS-FNA samples to histological material. Routine calculation of the proliferation index and subsequent grading of panNETs on cytologic material can be a useful tool in predicting the clinical behavior of these tumors and to guide further treatment and patient management.

360 HPV In-Situ Hybridization: Does Magnification Play a Role in Visual Evaluation?

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Background: Oropharyngeal cancers (OC) are rapidly increasing in incidence world-wide. Seventy percent of these cases are attributable to Human papillomavirus (HPV) comprising squamous cell carcinomas occurring mainly in non-smokers and non-drinkers. Determination of HPV status has strong diagnostic, prognostic, and therapeutic implications. Detection of HPV in metastatic cancer in neck lymph nodes may also be used to localize the primary within oropharynx, with a high degree of certainty. Determining the integration status of HPV by in situ hybridization (ISH) is cost effective and routinely utilized in clinical practice. However, the reliability of reporting is dependent on individual expertise and visual evaluation. In this study, we have analyzed the role of magnification in interpreting HPV ISH results.

Design: We retrospectively evaluated 63 small biopsies of primary OC and 34 FNA cell blocks of metastatic OC by HPV ISH for punctuate dot like nuclear positivity observed with low, intermediate or high magnifications, 10x, 20x or 40-60x. Positivity was assigned as 3+, 2+ and 1+ respectively. A single cell showing nuclear punctuate dot like staining was considered a positive result. A comparison was also made between initial interpretation and our review.

Results: Nineteen of 63 (30%) biopsies and 5 of 34 (14%) CB, positive at 10x magnification were considered 3+. Thirty one of 63 (49%) biopsies and 17 of 34 (50%) CB were positive at 20x magnification and were considered 2+. Thirteen of 63 (20%) biopsies and 12 of 34 (35%) CB showed positivity at 40-60x magnification and were considered 1+.

	10X (3+)	20X (2+)	40X -60X (1+)
Biopsies	19/63 (30%)	31/63 (49%)	13/63 (20%)
CB	5/34 (14%)	17/34 (50%)	12/34 (35%)

CB: Cell Block

A total of 22 biopsies and 16 CB were initially interpreted as negative but our review found them to be positive at 1+ magnification.

Conclusions: Magnification power plays a critical role in visual evaluation of HPV ISH. Although intermediate magnification (20x) reveals the most positivity (50%), it gives false negative results in a significant number of cases (20% biopsies and 35% CB). Failure to evaluate under higher magnification (1+) could confound the analysis and effect therapeutic decisions. The dot like punctuate staining may be very focal which requires careful visual determination of all tumor cells and this may have a learning curve for pathologists performing this evaluation. We recommend that HPV ISH slides should be interpreted at a high microscopic magnification, especially when most tumor cells appear negative.

361 Comparing HPV ISH and P16 in Assessing Metastatic Oropharyngeal Carcinoma

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Background: Background: The strong association of Oropharyngeal cancers (OPCs) with Human papilloma virus (HPV) infection, mainly HPV-16 and HPV-18, is now well recognized and well reported from various studies worldwide. HPV-positive tumors comprise a unique subset of head and neck squamous cell carcinomas (HNSCCs) that are different from HPV-negative tumors in respect to tumor differentiation, genetic changes, risk factors, risk stratification and prognosis. Detection of HPV status is now a standard practice in the pathological evaluation of HNSCCs. Strong correlations have been reported between diffuse nuclear and cytoplasmic p16 immunohistochemical staining (IHC) and HPV DNA detection by ISH. P16 IHC is used as a reasonable surrogate marker for high-risk HPV. Metastatic OPC may be the first clinical manifestation of the primary tumor and its evaluation may help in detecting the primary site. In this study we have compared the efficacy of HPV ISH and P16 IHC in the evaluation of metastatic OPCs.
Design: A total of 53 fine needle aspiration cell blocks (CB) from metastatic OPCs were evaluated with HPV ISH and P16 IHC. HPV ISH was interpreted as positive if a minimum of one tumor cell showed punctuate dot like nuclear positivity, P16 was interpreted as positive if 70% of tumor cells showed brown nuclear and cytoplasmic staining.10 CB from lung squamous cell carcinoma were taken as negative controls.
Results: Thirty four of 53 CB (64%) were positive for HPV ISH and 23 of 53 (43%) were positive for P16 IHC. 21 of 53 (39%) CB were positive for both HPV ISH and P16. The 10 CB from lung squamous cell carcinoma negative controls were all uniformly negative for HPV ISH and P16.

	HPV ISH	P16	Both
CB	34/53 (64%)	23/53 (43%)	21/53 (39%)

Statistical analysis in CB

	Sensitivity	Specificity	PPV	NPV	Accuracy
HPV ISH	64%	100%	100%	34%	69.8%
P16	43%	100%	100%	25%	52%

PPV: Positive predictive value, NPV: Negative predictive value

Conclusions: In comparing HPV ISH with P16 in cell blocks, we have determined that HPV ISH plays a more important role in determining HPV status in comparison to P16, as noted by a higher sensitivity, NPV and Accuracy rate. P16 staining is easier to recognize and evaluate on tumor cells in contrast to punctuate dot like positivity seen in HPV ISH which may be very focal and requires careful evaluation at a higher magnification. A definite learning curve exists for pathologists in evaluating HPV ISH since the task may be laborious and time consuming. The combination of both HPV ISH and P16 in assessing for metastatic OPC may be helpful in determining the HPV status of these tumors.

362 Detection of Soluble Mesothelin-Related Peptides as Diagnostic Markers of Malignant Pleural Mesothelioma Effusions: Comparison with Cytology

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Background: It has been reported that cytology (Cyt) allows the diagnosis of about 30% of malignant mesothelioma pleural effusions (MMPE). Recently, detection of soluble mesothelin (SM) levels has been proposed in order to help diagnosis of MMPE. In this study we assessed the diagnostic performance parameters (DPPs) of SM PE levels from a large cohort of patients and investigated whether SM level can improve Cyt diagnostic accuracy in routine clinical practice of malignant MMPE.
Design: We evaluated SM in 52 MMPE, 129 benign PE (BPE) and 94 non-MM pleural metastasis PE (MtsPE) by means of MesoMark ELISA kit (Fujirebio Diagnostic, Malvern, PA). DDPs were estimated through the ROC analysis. Youden's index was applied to obtain the best cut off level. The degree of correlation of SM in MMPE *vs* BPE and *vs* MtsPE was estimated by Diagnostic Odds Ratio (DOR) and by *P-value (P)* with Chi-square test. All specimens were subjected to routine cytology (Papanicolau staining).
Results: The median SM levels was significantly higher in MMPE (28.2 nM) than in BPE (3.2 nM) or MtsPE (3.8 nM). MMPE *vs* BPE yielded an AUC of 84.5 ($P<0.001$) whereas MMPE *vs* MtsPE yelded an AUC of 79.6 ($P<0.001$). The cut off level in MMPE was estimated in 9.30 nM at which value we established Se=75%, Sp=93% for MMPE *vs* BPE and SP=81% for MMPE *vs* MtsPE. We found SM positive cases (≥ cut off) in 38/52 (73%) MMPE, in 9/129 (7%) BPE (DOR=40, $P<0.001$) and in 18/94 (19%) MtsPE (DOR=13, $P< 0.001$).
Cyt was negative in 29/52 (56%) of MMPE (16 epithelioid, 8 sarcomatoid, 2 biphasic, 2 desmoplastic, 1 papillary), among which SM was positive in 20/29 (69%) cases (14 epithelioid, 3 sarcomatoid, 2 biphasic, 1 papillary).
Cyt was positive in 15/52 (29%) of MMPE, (12 epithelioid, 1 sarcomatoid, 2 biphasic), among which SM was negative in 4/15 (27%) cases (all epithelioid).
Finally, in 8/52 (15%) MMPE (7 epithelioid, one papillary) Cyt diagnosis was suspicious and SM was found positive in 7/8 (88%) cases (6 epithelioid, 1 papillary).
Conclusions: SM detection in MMPE may provide additional diagnostic value to Cyt. Combination of SM and Cyt have major diagnostic possibilities if both tests may be performed together.
SM test may be incorporated into clinical practice of MMPE from patients suspicious for malignant mesothelioma.

363 ER, PR, and Her2 Immunocytochemistry on Cell-Transferred Cytologic Smears of Breast Carcinoma – A Study with Comparison to Formalin-Fixed Tissue

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Background: The presence of ER, PR, and Her2 can be used to predict response to treatment in patients with primary or metastatic breast carcinoma. Fine needle aspiration (FNA) can provide cellular material which may be used for such analysis. Formalin-fixed cell blocks (CBs) have been used for this purpose; however, CBs sometimes lack adequate cellularity even when the direct smears are highly cellular. The aim of this study is to assess the reliability of ER, PR, and Her2 status as demonstrated by immunocytochemistry (ICC) on slides made from alcohol-fixed direct smears using the cell transfer (CT) technique.
Design: A computerized search of the laboratory information system was performed. FNA cases diagnosed as primary or metastatic breast carcinoma in which ICC for ER, PR, and Her2 had been performed either on a CB or a concurrent biopsy were identified over an 18 month period (January 2010 through July 2011). All correlating cytology and surgical pathology reports were reviewed as well as selected slides. ICC for ER, PR, and Her2 was performed on alcohol-fixed direct smears using the CT technique. The results were compared to those reported for the corresponding CB or biopsy.
Results: A total of 47 FNA specimens from 46 patients were included in the study. ICC for ER, PR and HER2 were scored as recommended by CAP/ASCO guidelines. ICC results were excluded from the study if the CT smear contained less than 50 cells. Correlation between the ICC on the CT smears and the corresponding CB or biopsy revealed a sensitivity rate for ER, PR, and Her2 of 95%, 90%, and 88% respectively with a specificity of 100% for all 3 markers. There were 4 cases in which the ICC for Her2 on the CT smear was scored as 2+ (equivocal), of which 2 were negative and 2 were equivocal on the corresponding CB or biopsy. There was 1 case in which the ICC for Her2 on the CT smear was scored as 1+ in which the corresponding biopsy was equivocal for Her2. These 5 cases were excluded from calculation of sensitivity and specificity for Her2.
Conclusions: ICC performed on FNA smears using the cell transfer technique is useful in the assessment of ER, PR, and Her2 status, especially when the direct smears are highly cellular and the CB lacks adequate cellularity. The specificity is 100% for all 3 markers and the sensitivity is high with a rate of 95%, 90%, and 88% for ER, PR and HER2 respectively.

364 Hologic Thinprep Imaging System for Routine Urine Screening: Evaluation of Screening Time and Diagnostic Comparison

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Background: Automated screening has become essential in decreasing screening time and calculated workload for cytotechnologists' evaluation of Pap test slides. We propose the novel use of the same Hologic Thinprep Imaging System (Imager) for evaluation of urine with assessment of screening time and diagnostic outcome in comparison to routine Thinprep full manual review (FMR).
Design: In this prospective study, urine Thinprep pap stained slides were processed for diagnostic evaluation with consecutive Thinprep slides using the Hologic Imaging system pap staining kit (n=86). Clinical records for cytotechnologists and cytopathologists' diagnoses were reviewed with comparison to timed re-review of the same Thinprep urine slide by FMR (EMK) and Thinprep Imager-stained slides (KF, EMK). Routine practice prompts a FMR when the Thinprep Imaging system rejects screening of the slide or if a diagnostic category of atypical or greater is seen in the Imager designated 22 fields of view (FOV).
Results: Results indicate concordant diagnoses in 78% (67/86) up to 91% (78/86) if Imager-rejected samples are included. Categorical diagnostic changes based on the Imager- stained slide include negative to atypical (2); atypical to negative (3); non-diagnostic to negative (2); and negative to non-diagnostic (1). Cytotechnologist timed 22 FOV of 52 Imager-stained slides averaged 48 seconds per case; versus FMR of slides rejected by the Imaging system (11 cases, 12 sec/case) and FMR of 6 cases triggered by cytologic atypia or worse (84 sec/case). Cytopathologist full slide screening (n=86) of Thinprep urine slides was 48 sec/case versus 84 sec/case in Imager-stained urine slides.
Conclusions: Our data shows the advantages of using Hologic Thinprep Imaging System to decrease cytotechnologist screening time with comparable diagnostic outcome. In a large volume setting, where the majority of urine samples are negative, further study will be necessary to determine if the added testing costs is outweighed by the benefit of automated imaging system reductions in cytotechnologist time and calculated workload.

365 The Value of Repeated Fine-Needle Aspiration Biopsy in an Academic Community Hospital after the Bethesda System

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Background: Follow-up of thyroid nodules with repeated fine-needle aspiration biopsies (rFNA) is recommended in nondiagnostic (ND) samples and in cases of atypia of unknown significance (AUS)/follicular lesions of uncertain significance (FLUS), however, the impact of this approach is generally unexplored. We evaluated the risk of neoplasia (RN) and malignancy (RM) in rFNA.
Design: All FNA from Jan/04 to Dec/10 were reclassified according to Bethesda System: ND, benign (B), AUS/FLUS, suspicious for follicular neoplasm (FN), suspicious for malignancy (SM) and malignant (M). Patients with one FNA (1FNA) and with rFNA were compared according to the worst diagnosis in the first FNA (fFNA) or in the rFNA. Surgical pathology (SP) and clinical follow-up were retrieved.

Results: In 480 patients (F:M=7:1, average age 53), 70 (14.6%) had rFNA. Average number of rFNA was 1.3±0.8. A total of 125 (26%) had a thyroidectomy (21.4% in the rFNA and 26.8% in 1FNA - p=0.3). Diagnoses upon fFNA in rFNA group were ND in 10 (14.3%), B in 49 (70%), AUS/FLUS in 8 (11.4%) and FN/SM in 3 (4.3%). In B group rFNA changed in 3 patients (6.1%) (2 AUS/FLUS, 1 FN) and 11 patients (22.4%) had SP follow-up: 1 follicular adenoma (FA) and 10 benign non-neoplastic lesion (BN), including 1 patient with AUS/FLUS at rFNA. In ND group rFNA changed in all patients: 9 (90%) to B and 1 (10%) to M – SP confirmed papillary carcinoma (PC). In AUS/FLUS group rFNA changed to B in 3 (37.5%) and ND in 1 (12.5%), none with SP. AUS/FLUS rFNA was unchanged in 4 (50%) – SP available in 3: 1 PC, 1 papillary microcarcinoma (PMC) and 1 BN. rFNA changed to B in the 3 patients of FN/MS group, one with BN at SP. Diagnoses in 1FNA group with SP follow-up were ND in 1 (0.9%), B in 60 (54.5%), AUS/FLUS in 15 (13.6%) and FN/SM/M in 34 (30.9%). The 1 ND was BN at SP. In 1FNA B group SP confirmed 7 incidental PMC (11.7%) and 1 FA (1.7%). In 1FNA AUS/FLUS group SP showed 1 FA (6.7%), 1 PC (6.7%) and 1 PMC (6.7%). In 1FNA FN/SFN/M group SP showed 18 PC (52.9%), 6 PMC (17.6%), 1 follicular and 1 medullary carcinoma. RM was 9.1% for all ND FNA. General RN was 9.1% in rFNA B group, 15% in the 1FNA B, 66% in rFNA AUS/FLUS, 20% in 1FNA AUS/FLUS and 82.4% in 1FNA FN/SM/M.

Conclusions: Our data support the recommendation of rFNA in ND category. A repeated diagnosis of AUS/FLUS increased the general RN from 20% to 66% (p=0.1). A B fFNA diagnosis had a 4% chance of changing upon rFNA, and a virtually null RM.

366 Immunohistochemical Analysis of EZH2 and E-Cadherin Expression in Pancreatic Adenocarcinoma in Fine Needle Aspiration

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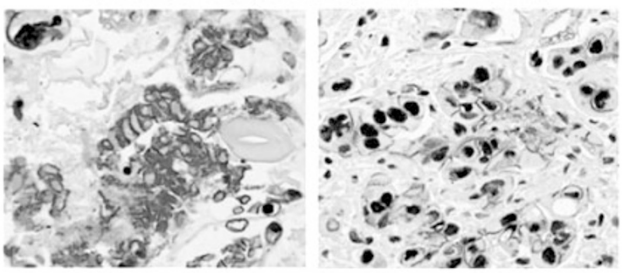
Background: Pancreatic adenocarcinoma (PAC) is the fourth deadliest cancer in the US. Endoscopic ultrasonography-guided fine needle aspiration (EUS-FNA) has improved PAC detection by obtaining diagnostic tissue that may be the only available in the inoperable patients. Recent studies show that partial or complete loss of E-cadherin (EC) is an independent poor prognostic indicator of PAC, related to anti-VEGF drug resistance. Similarly, nuclear accumulation of enhancer of zeste homolog 2 (EZH2), a histone methyltransferase that mediates increased invasiveness and metastasis by silencing downstream targets including EC, is a hallmark of poorly differentiated PAC. Depletion of EZH2 in vitro has been shown to sensitize cancer cells to chemotherapy. The present study aims to determine EC and EZH2 status in PAC in order to provide biomarkers for prognosis and potential treatment.

Design: IHC was performed on paraffin sections of EUS-FNA cell blocks from 38 PAC cases selected from 2009-2011 Cytopathology files. GI pick-ups served as an internal control. The intensity of EZH2 and EC expression in PAC and normal GI pick-ups were scored by a four tier grading system: negative (-), weak positive (+), moderate positive (++ to +++) and strong positive (++++).

Results: In 33 PAC cases, EZH2 demonstrated variable nuclear reactivity focally or diffusely. In GI pick-ups, EZH2 showed diffuse moderate nuclear reactivity. EC demonstrated strong membranous expression in normal epithelium and partial or complete loss in the areas of PAC. The majority of cases with poorly differentiated PAC, especially single tumor cells, showed near or complete loss of EC, while the nuclear reactivity of EZH2 was focally or diffusely increased (Fig 1). However, not all single tumor cells showed strong EZH2 expression. Five PAC cases showed minimal or no EZH2 expression but decreased EC expression, and 2 showed complete loss of EC focally.

Double IHC for EC (red) and EZH2 (brown) in Two PAC Cases



Conclusions: IHC for EZH2 and EC can be successfully performed on EUS-FNA of PAC. Our results indicate that EZH2 and EC are not always inversely expressed, and the EZH2 expression level does not always correlate with the level of tumor differentiation. Therefore, analysis of EZH2 and EC expression may be helpful in prognostic stratification and treatment decisions in PAC.

367 Combination of Urine Cytology and FGFR3 Mutation for the Diagnosis of Urothelial Carcinoma

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Background: There were 70,980 new cases of urinary bladder carcinoma (BC) with 14,330 deaths in the United States in 2011. Urothelial carcinoma (UC) is the most common type, it primarily presents as a nonmuscle-invasive tumor (NMI). 80% of UC patients suffer recurrence within 2 years of initial treatment. Urine cytology is the main screening and follow-up tool, but sensitivity (66.6%) is not high for low-grade (LG) tumors. FGFR3 is a tyrosine kinase important in differentiation and proliferation. 80% of patients with NMI tumor have FGFR3 mutations, this is associated with improved prognosis. 17% of HGUCs have FGFR3 mutations, the presence of mutation does NOT impact prognosis. A simple FGFR3 mutation assay of bladder washing could increase diagnostic sensitivity when combined with urine cytology. This study demonstrates feasibility of such an assy in correlation with urine cytology and surgical histology of the bladder.

Design: Fifteen fresh bladder washings from 2011 were spun-down after cytology slide preparation, the pellets stored at -20^{0}C. Only specimens with a high urothelial cellularity were tested. All diagnoses were reviewed by two cytopathologists. DNAs were extracted from the cell pellets, Exons 10 and 15 were PCR amplified, then sequenced with BigDye Terminator Cycle Sequencing Kit (v1.1). The capillary gel electrophoresis was on an ABI PRISM 3100; mutations were analyzed with Mutation Surveyor Software (Soft Genetics).

Results: None of the specimens with only reactive (n=6) or atypical (n=2) urothelial cells showed mutations. None of the ileal conduit urine samples (n=2) with degenerated urothelial cells suspicious for malignancy showed mutations. Two of three cases with atypical urothelial cells suspicious for neoplasm in cytology had biopsies which showed high grade papillary urothelial carcinoma; one invasive and the other non-invasive. The latter case revealed a point mutation (G382R). Two cases proven by biopsy to be high grade UC or mixed high grade carcinoma were easily diagnosed as UC in cytology; however neither showed an FGFR3 mutation. An intronic mutation near exon 15 was identified.

Conclusions: Our results indicate that bladder washings can be suitably processed for FGFR3 mutation analysis in conjunction with cytopathological analysis. Extension of this study with more sensitive point-mutation specific assays in combination with urine cytology could increase the diagnostic sensitivity of detection of NMI UC.

368 The Value of Second Opinion Review of Cytologic Specimens from the Head and Neck

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Background: As a quality assurance practice, review of cytopathologic specimens from the original institution can refine the cytologic diagnosis and guide clinical management of patients referred for surgical evaluation of lesions in the head and neck. Routine review of cytologic specimens can allow for the identification of diagnostic errors, obviate repeat fine needle aspiration, and guide proper treatment. The frequency of clinically significant diagnostic discrepancies in cytopathology at a large referral hospital is unknown.

Design: All cytopathologic specimens submitted by the Department of Otolaryngology-Head and Neck Surgery to the Division of Cytology between January 1, 2000 and May 1, 2011 were reviewed. The diagnosis made by the original pathologist was compared to that rendered at the referral hospital. A diagnostic discrepancy was defined as any change in diagnosis that would likely result in significant modification of clinical management.

Results: A diagnostic discrepancy was identified in 89 of 512 of cases (17%). Specimens prone to diagnostic discrepancy include thyroid and lymph node aspirates. The most common discrepancy was the change from an "atypical" or "suspicious" diagnosis rendered on a thyroid aspirate to a definitive diagnosis of papillary, medullary, or anaplastic thyroid carcinoma. Lymph node aspirates with a "benign," "atypical," or "suspicious" diagnosis were frequently given a frankly malignant diagnosis on second review. Histologic evaluation confirmed the second opinion diagnosis in every discrepant case where a cytologic diagnosis of thyroid carcinoma or malignancy within a lymph node was rendered. Definitive cytologic diagnosis in these cases frequently obviates repeat fine needle or excisional biopsy and facilitates definitive surgical or medical management.

Conclusions: Routine review of cytologic specimens for patients referred for surgical evaluation of lesions in the head and neck results in a potentially clinically significant change of diagnosis in 17% of cases. Even "minor" changes in interpretation result in significant modifications of surgical planning.

369 Utility of Carbonic Anhydrase-IX in the Diagnosis of Metastatic Conventional Renal Cell Carcinoma by Fine-Needle Aspiration Biopsy

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Background: Expression of the commercially available antibody against Carbonic anhydrase-IX (CAIX) has been shown to be useful in the diagnosis of metastatic conventional renal cell carcinoma (cRCC) in surgical pathology specimens, but studies demonstrating its utility in cases of metastatic cRCC from cell-block sections of aspiration biopsies are limited. The objective of the current study was to compare CAIX expression with other immunohistochemical markers routinely used in the diagnosis of metastatic cRCC (CD10, and Vimentin) in cell block material from fine-needle aspiration biopsies (FNABs), and to asses its utility in confirming the diagnosis of metastatic cRCC in cytologic specimens.

Design: Thirteen metastatic RCC specimens from 12 patients were immunostained with CAIX, CD10, and Vimentin. The immunoreactivity results were compared. The metastatic sites included pancreas (4), thoracic lymph nodes (4), bone (3), soft tissue (1), and pleural fluid (1). The patient ages ranged from 57 to 86 years old, (mean age of 67). The commercially available CAIX antibody, clone NB100-417 (Novus Biological, Littleton, CO), was used in this study. Immunohistochemistry (IHC) staining was performed and immunoreactivity was graded as 0, no tumor cells immunoreactive (IR); 1+, 1% to 25% IR; 2+, 25% to 50% IR; and 3+, greater than 50% IR.

Results: Eleven of 13 (85%) cases showed 3+ strong and diffuse membranous staining with CAIX; 12 of 13 (92%) cases showed similar 3+ IR with CD10; and 9 of 12 (75%) cases showed 3+ strong and diffuse cytoplasmic IR for Vimentin. In 1 case where CAIX IR was 1+, both CD10 and Vimentin showed 3+ IR; while in the other case where CAIX IR was 1+, CD10 showed 0 IR and Vimentin showed 1+ IR. Morphologically,

12 of 13 metastatic cRCCs showed small to intermediate sized nuclei with relatively inconspicuous nucleoli, and 1 case showed larger nuclei with prominent nucleoli. In the 2 cases where CAIX showed 1+ IR, the tumor cells were intermediate in size with inconspicuous nucleoli. In the 1 case with prominent nucleoli, both CAIX and CD10 showed 3+ IR, while Vimentin showed 1+ IR.

Conclusions: The majority of cases in this study showed strong and diffuse expression of CAIX, and with the exception of 1 case the expression of CD10 and Vimentin was comparable. This study suggests that CAIX is a useful marker in addition to CD10 and Vimentin to confirm the diagnosis of metastatic cRCC in cytologic cell block material from various anatomical sites.

370 Comparison of HER2 Gene Status Determination by HER2 Dual ISH DNA Probe Cocktail Assay Performed on Cell Block Material to Immunohistochemistry (IHC) and Fluorescence In Situ Hybridization (FISH) Performed on the Corresponding Histologic Specimen

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Background: The human epidermal growth factor receptor 2 (HER2) gene is amplified in 18% to 20% of breast cancers. HER2 gene amplification status is important for therapeutic decision making, and The American Society of Clinical Oncology (ASCO) Tumor Marker Guidelines Panel has recommended routine testing of HER2 on newly diagnosed and metastatic breast cancers. Cell block preparations of cytologic material are a valuable tool for determining HER2 gene amplification status on metastatic lesions, which are often sampled by fine needle aspiration (FNA) alone. The INFORM HER2 Dual ISH DNA Probe Cocktail assay by Ventana Medical Systems, Inc., uses two color chromogenic in situ hybridization (CISH) to determine HER2 gene status with light microscopy. This allows for the identification of patients who would benefit from Herceptin (trastuzumab) therapy.

Design: Cytologic samples obtained from fresh resection specimens with biopsy proven invasive mammary carcinoma were processed into three types of cell blocks (Cellient, thrombin, and formalin). The INFORM HER2 Dual ISH DNA Probe Cocktail assay was performed on the cell blocks. The ratio of HER2 to chromosome 17 signals was calculated to determine the HER2 gene amplification status. The HER2 gene amplification status of each biopsy or resection specimen, as determined by IHC and/or FISH, was compared to the results obtained from the assessment of cell blocks analyzed by the HER2 Dual ISH DNA Probe Cocktail assay.

Results: Comparison of HER2 gene status by Dual ISH DNA Probe Cocktail Assay on cell block material showed 100% correlation with the HER2 gene status determined by either IHC or FISH on histologic specimens. Through ongoing process optimization tailored toward cell block material, enumerable signals and ratios were calculated on eight cell blocks, which included three Cellient cell blocks, two formalin cell blocks, and three thrombin cell blocks. All the cases were HER2 gene amplification negative. No false-positive results occurred.

Conclusions: While further validation and study is underway, preliminary results show that breast carcinoma HER2 gene amplification status can reliably be determined on cell block material using The INFORM HER2 Dual ISH DNA Probe Cocktail assay.

371 Biliary Stent-Related Atypia Can Be Reliably Distinguished from Adenocarcinoma on Common Bile Duct Brushings Using a Limited Number of Cytologic Features

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Background: Although it is widely accepted that cytologic atypia secondary to biliary stent is a potential pitfall in pancreatobiliary exfoliative cytology, no systematic study has been undertaken to identify the cytologic features that best distinguish these entities.

Design: A retrospective search of our archives for a four year period revealed 14 cases of bile duct brushings with biliary stents classified as either atypical or suspicious for malignancy, each with greater than 6 months of benign clinical follow-up. 15 comparison cases of bile duct brushings classified as positive for malignancy with histologic confirmation of adenocarcinoma were also identified. Cytologic features such as nuclear enlargement, nuclear contour, nuclear overlap, chromatin distribution, N/C ratio, anisonucleosis (3x size variation), mitoses, acute inflammation, disorganization, necrosis, prominence of cell borders, presence of single atypical cells, and of two distinct cell populations were assessed on Papanicolaou-stained conventional smears and liquid-based preparations. Fisher's exact test was used to determine statistical significance.

Results: We identified 5 cytologic features which achieved statistical significance: anisonucleosis ($p=0.0422$), atypical single cells ($p= 0.0001$), and two distinct cell populations ($p= 0.0007$) each favored malignancy, while distinct cell borders ($p= 0.0018$) and acute inflammation ($p= 0.0035$) favored benign. No other feature was statistically significant. Using a simple diagnostic algorithm incorporating only these 5 features, we were able to correctly classify 12/14 benign and 15/15 malignant cases, with a sensitivity of 88.2%, a negative predictive value of 85.7% and a specificity and positive predictive value of 100% each. In addition, on blinded review of the same cases with a focus on these criteria and enforcing a binary diagnostic classification, we correctly classified 13/14 benign and 14/15 malignant cases.

Conclusions: By using a combination of 3 markers of malignancy and 2 favoring benignity, we were able to correctly classify 27 of 29 cases. These findings suggest that most bile duct brushings from patients with biliary stents can be definitively and correctly classified as either benign or malignant using a combination of features including anisonucleosis, single atypical cells, binary cell population, distinct cell borders, and acute inflammation. Collection of additional cases to serve as a validation set is ongoing.

372 Establishing Optimal Digital Scanning Parameters of 3-D Gynecological Virtual Images: *Follow-Up Study*

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Background: Virtual microscopy (VM) in cytology has been limited by the inability of focusing through the 3 dimensional (3D) cell clusters of cytological specimens acquired with single focal plane (2D images). Limited information exists regarding the optimal scanning parameters for 3D scanning. We previously established an optimal scanning interval of 1 micron. The goal of this study is to determine the optimal number of scanning focal plane levels.

Design: Twenty SurePath™ slides were scanned at 40X magnification at 1 micron with 7 focal planes (Group 1),5 focal planes (Group 2) and 3 focal planes (Group 3) using iScanCoreo Au scanner (Ventana, AZ, USA). 60 virtual images (VI) were produced. A cytopathologist, cytotechnologist, pathology resident and a cytotechnology student used BioImagene's Image Viewer (version: 3.0.0.0 – RC8) and diagnosed pre-annotated cells of group 1, 2 and 3. They recorded the focal plane level at which they were confident of the diagnosis. Subsequently, they diagnosed the corresponding 20 glass slides (Group 4) using conventional Light Microscopy (LM). Participants selected standard Bethsda diagnostic categories. We evaluated interobserver reliability using kappa statistics.

Results: The interobserver reliability was found to be highest for the glass slides (97%) followed by VI scanned at 3 focal plane levels (80%), 7 focal plane levels (78%) and 5 focal plane levels (76%). See table 1.

Table 1: Results	Glass slides	7 focal plane levels	5 focal plane levels	3 focal plane levels
Interobserver reliability	97%	78%	76%	80%
Sensitivity	100%	92%	89%	95%
Specificity	100%	100%	96%	98%
PPV	100%	100%	97%	97%
NPV	100%	93%	91%	95%
Total number of focal planes used to diagnose the virtual images	-	7	5	3
Average file size	-	9.5 GB	6.8 GB	4.2 GB

Conclusions: VI scanned with 3 focal plane levels at 1 micron interval has potentially the highest interobserver reliability, sensitivity, NPV and the lowest file size. This finding will guide a larger confirmatory study to establish the optimal scanning focal plane and interval of gynecologic specimens.

373 Pancreatic Cyst Fluid Cytology and Carcinoembryonic Antigen (CEA) Level Obtained by Endoscopic Ultrasound Guided- Fine Needle Aspiration: Which Is Better at Identifying High Grade Dysplasia/Invasion in Intraductal Papillary Mucinous Neoplasms?

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Background: Endoscopic ultrasound guided fine needle aspiration (EUS-FNA) is routinely used at the Moffitt Cancer Center to sample suspected pancreatic intraductal papillary mucinous neoplasms (IPMNs) in order to guide surgical management. Previously, we demonstrated that cyst fluid CEA level is not a reliable predictor of IPMN grade of dysplasia. Here, we evaluated the EUS-FNA cytological diagnoses in the same population to determine if cytology is more effective at identifying malignant IPMN.

Design: Using an IRB-approved comprehensive IPMN cancer database we selected all patients who had previously undergone EUS-FNA of the cystic mass with cyst fluid CEA measurement and cytology. IPMNs were classified into low grade dysplasia (LG), moderate dysplasia (MD), high grade dysplasia (HG) or invasive carcinoma (INV). The cytology reports were prospectively reviewed, and each cytology specimen characterized as malignant (HG or INV) or nonmalignant (LG or MD). One-way ANOVA was performed to examine the difference in CEA levels among pathologic groups. The cytology results were correlated with the histology to calculate sensitivity, specificity, and positive (PPV) and negative predictive values (NPV).

Results: We identified 50 patients (22 males, 28 females). There were 9 LG, 19 MD, 15 HG and 7 INV. The fluid CEA levels and corresponding histological diagnoses are summarized in Table 1. No statistically significant difference was found in CEA levels among diagnoses ($p=0.62$). Cytology identified 6 of 7 INV as malignant (3 INV and 3 HG), and 3 of 15 HG as malignant (3 HG). 1 INV had indeterminate results. None of the 28 IPMN with LG or MD were classified as malignant. Using this two-tiered system, the sensitivity, specificity, PPV and NPV for the cytological detection of malignant IPMN were 45.5%, 100%, 100% and 70%, respectively. The sensitivity for the detection of INV alone was 85.7%.

Conclusions: Cyst fluid cytology more accurately predicts malignant IPMN than CEA level and has high specificity. Our data demonstrate that a pre-operative cytological diagnosis of malignant IPMN (HG or INV) reliably correlates with the final histological diagnosis.

Table 1: Fluid CEA Levels and Corresponding Histological Diagnoses

Histological Diagnosis	CEA ranges (ng/ml	Mean CEA, ng /ml	Median CEA, ng/ml
LG (N=9)	37.9-4541	1261.3	458
MD (N=19)	7.6- 90,100	6433.6	201
HG (N=15)	47.2- 136,441	12264	373.9
INV (N=7)	140- 1866	462.57	200

374 The Bethesda System for Reporting Thyroid Cytopathology: A Single-Institution Retrospective Analysis of 2,479 Cases

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Background: The Bethesda System for Reporting Thyroid Cytopathology (TBSRTC) provides a uniform diagnostic nomenclature and classification system, including 6 major categories - benign (B), atypia of undetermined significance (AUS), follicular neoplasm (FN), suspicious for malignancy (SM), malignancy (M) and non-diagnostic (ND), for the interpretation and reporting of thyroid fine-needle aspirates (FNA). We retrospectively applied the TBSRTC terminology to thyroid FNA cases previously diagnosed at our institution in order to assess the implied risks of malignancy for each of the 6 major diagnostic categories.

Design: A computer search of our anatomic pathology information system was performed for the 7-year period from 2003 to 2010 and all thyroid FNA cases were identified. Five cases of parathyroid hyperplasia were excluded from this analysis. The FNA diagnoses were reclassified according to TBSRTC and correlated with the available follow-up thyroid surgical pathology reports. The risk of malignancy (malignant tumors excluding papillary microcarcinoma) and the risk of neoplasm (malignant tumors including papillary microcarcinoma plus adenomas) were calculated for each diagnostic category.

Results: A total of 2,479 thyroid FNA aspirates were performed during the 7-year period encompassed by this study. The cytologic diagnoses were as follows: 1,751 cases (71%) were B, 311 cases (13%) were AUS, 72 cases (3%) were FN, 58 cases (2%) were SM, 181 cases (7%) were M, and 101 cases (5%) were ND. Surgical pathology follow-up was obtained in 348 cases (14%). For the subset of cases with surgical pathology follow-up, the risk of a neoplasm for each diagnostic category was as follows: B 40%, AUS 71%, FN 89%, SM 88%, and M 99%. The risk of malignancy for each category was B 4%, AUS 15%, FN 24%, SM 88%, and M 99%.

Conclusions: The TBSRTC classification system stratifies the risk of thyroid neoplasia and malignancy in a manner that is simple, understandable and useful for therapeutic decision-making. Follow-up recommendations are appropriate given the significant differences in the risk of neoplasia and malignancy associated with each of the TBSRTC diagnostic categories.

375 Retrospective Review of False-Negative Thyroid FNAs

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Background: Fine needle aspiration (FNA) cytology is a cost-effective procedure that provides specific diagnoses rapidly with minimal complications. A major utility of FNA is to prevent unnecessary surgery in patients whose findings are unequivocally benign. False negative diagnoses are reported at varying frequencies in the literature. The purpose of this study was to systematically analyze false negative thyroid FNAs (FN-TFNA) and determine the impact patient outcome.

Design: Medical records of 316 consecutive patients over a four-year period (2007-2011) were reviewed to identify cases in which a prior FNA was diagnosed as "negative for malignancy" in the same nodule that had a subsequent malignant surgical pathology diagnosis. Two experienced cytopathologists re-adjudicated the FNAs to determine whether a diagnostic interpretative error occurred. For a subset of cases with sampling error, re-review of the surgical specimen was performed. Various clinical parameters and pathologic data were recorded and analyzed.

Results: Nine malignant nodules in 8 patients with FN-TFNA were identified. The nine malignant nodules include 6 follicular variants of papillary thyroid carcinoma and 3 follicular carcinomas. The tumors were organ confined in seven of eight patients. Only one of eight patients had lymph node involvement at the time of thyroidectomy. Seven of eight patients are alive and free of disease on follow-up studies (avg. follow-up 25 months). One of eight patients is alive with radiographic evidence of metastatic disease. The average time from the FN-TFNA to diagnosis was 11.2 months (2-29 months). Adjudicated diagnoses agreed with the negative FNA diagnosis in five of nine cases. In the other four cases, two were adjudicated to be atypical and two were adjudicated to be suspicious for carcinoma.

Conclusions: FN-TFNAs occur in nodules harboring low-grade tumors and the majority of the time are low stage at diagnosis. The delay in diagnoses did not directly lead to adverse clinical outcomes in our series, as the only patient with metastatic disease was promptly diagnosed due to the clinical behavior or the tumor. FN-TFNAs encompass both interpretive and non-interpretive errors. Non-interpretive errors are thought to be due to sampling error, tumor heterogeneity or terminal differentiation of tumor cells. Review of FN-TFNA due to interpretative error can be a valuable educational tool. Although FN-TFNA is not desired, clinical outcomes in our series suggest that thresholds should not be lowered to the point of over interpreting mildly atypical aspirates.

376 EZH2, a Unique Marker of Malignancy in Effusion Cytology

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Background: Distinguishing reactive mesothelial cells from metastatic disease in effusion cytology can be challenging at times. We currently use of a panel of immunocytochemical (ICC) markers for select cases including MOC-31 and BerEp4, but difficulties still exist, especially when the questionable cells are scarce. Enhancer of zeste homologue 2 (EZH2), a member of the Polycomb group of genes, plays important roles in epigenetic silencing and cell cycle regulation and is upregulated in a wide variety of malignancies. Thus, we hypothesized that EZH2, which to our knowledge has not yet been reported on cytology material, might serve as a unique marker of malignancy in morphologically equivocal effusion specimens.

Design: A retrospective search over a two year period ending 6/30/2011 was performed and clear cut effusion cases were selected as follows. Diagnoses were confirmed by review of cytomorphology and also had to be supported by clinical history, radiology, and pathology as applicable. For benign cases, those with a history of malignancy or a significant concern for malignancy were specifically excluded. Cases without sufficient cells of interest remaining on cell block were also excluded. The expression of EZH2 was assessed by ICC after optimization for cytologic material including antigen retrieval with EDTA and using a 1:10 dilution of monoclonal anti-EZH2 (Biocare Medical, Concord, CA). An ICC staining assessment method that has previously been applied to cytology effusions was adopted where a positive result was defined as appropriate (i.e. nuclear) staining in at least 5% of the cells of interest.

Results: In total, 27 effusions were analyzed including 9 benign (5 pleural and 4 ascitic) and 18 malignant (9 pleural, 8 ascitic, and 1 pericardial) with primary sites as follows: 3 lung, 3 breast, 3 ovary, 5 uterus, 1 stomach, 1 pancreas, 1 kidney, and 1 of unknown primary. All malignant cases were metastatic carcinomas except for one, an epithelioid endometrial stromal sarcoma. The benign cases were all negative for EZH2, and 16 of 18 malignant effusions were positive; the pancreatic and renal primaries were the outliers. As a solitary ICC marker, EZH2 exhibited a sensitivity of 89% and a specificity of 100%.

Conclusions: EZH2 functioned as a unique and accurate marker of malignancy in this series of effusions. It demonstrated comparable sensitivity and improved specificity relative to published data for MOC-31 and BerEp4. After confirmation in larger studies, EZH2 may prove to be of great value in routine diagnostic work.

377 Diagnostic Value of FNA Processed by ThinPrep for Assessment of Axillary Lymph Node Status in Patients with Invasive Carcinoma of Breast

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Background: Ultrasound-guided FNA has been widely utilized as an important modality in determination of axillary lymph node status during the initial staging and subsequent management of patients with invasive breast carcinoma. Tranditionally, the aspirates are processed as conventional smears. However, in the current study, we evaluated the utility of the use of ThinPrep as a more standard method for detection of axillary lymph node metastasis of invasive breast carcinoma.

Design: A Computer SNOMED Search from the file at our institution between 01/20003 and 08/2011 was conducted to identify patients with invasive breast carcinoma who were worked-up by FNA of axillary lymph node and followed by axillary lymph node dissection. We retrieved a total of 209 FNAs processed by ThinPrep including 193 and 16 diagnostic and non-diagnostic specimens, respectively. The 193 diagnostic specimens consisted of 168 invasive ductal carcinoma (IDC), 15 invasive lobular carcinoma (ILC) and 10 mixed carcinoma(IDC and ILC). Using the histology diagnosis as the golden standard, the diagnostic parameters were determined. Slides from cyto-histologic discrepant cases were re-reviewed.

Results:

ThinPrep for assessment of axillary lymph node status in patients with invasive breast carcinoma

Parameters	IDC	ILC/Mixed carcinoma	Total
True positive	110	16	126
True negative	32	4	36
False positive	0	0	0
False negative	26	5	31
Sensitivity (%)	81	76	80
Specificity (%)	100	100	100
PPV (%)	100	100	100
NPV (%)	53	44	54
Accuracy (%)	84	80	84

PPV: positive predictive value; NPV: negative predictive value.

Using FNA processed by ThinPrep for assessment of axillary lymph node status in patients with invasive breast carcinoma:

1) Ninety-two percent of the specimens are adequate for cytologic diagnosis.

2) Both diagnostic sensitivity and accuracy for detection of lLC/mixed carcinoma (76%, 80%) are slightly lower than that of IDC (81%, 84%).

3) NPV for detection of IDC (53%) is greater than that of ILC/mixed carcinoma (44%).

4) Both specificity and PPV reach 100% regardless the types of invasive breast carcinoma.

5) Sampling error is the major factor contributing to false negative cytolgy interpretation.

Conclusions: Compared to the previously reported data on FNA with conventional smear preparation, FNA processed by ThinPrep offers a compatible diagnostic sensitivity while reaching 100% of specificity. It is an efficient and more convenient method for assessment of axillary lymph node status in patients with invasive breast carcinoma.

378 Malignancy Risk Is Similar for Solitary and Multiple Nodules in Hurthle Cell-Predominant Thyroid Fine Needle Aspirations

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Background: Hurthle cell predominant (HC) thyroid fine-needle aspirations (FNAs) are problematic. FNAs diagnosed as "Suspicious for a Hurthle Cell Neoplasm" (HUR) have high sensitivity but low specificity for malignancy, especially since Hurthle cells are common in reactive and hyperplastic settings. The Bethesda System (TBS) indicates that the presence of multiple nodules may justify classifying HC FNAs as "Atypia of Undetermined Significance" (AUS) rather than HUR. We set out to address whether this approach is justified.

Design: Cytology records were searched for HC thyroid FNAs and cases were correlated with ultrasound (US) and surgical pathology reports. All FNAs were endocrinologist performed with US-guidance and were processed by ThinPrep (Hologic, Marlborough, MA) liquid-based preparation.

Results: 165 HC FNAs from 146 nodules were diagnosed as: Benign (5), AUS (40), HUR (116), Suspicious for Malignancy (3), and Malignant (1). By US, the median size of all targeted nodules was 1.9 cm. 21 nodules were solitary, while 122 nodules were in the setting of multiple (≥2) nodules. Results for 99 resections (60 benign, 39

malignant) were reviewed. Of the 60 benign nodules, US showed 49 (82%) associated with multinodular disease with median size of targeted nodules of 1.9 cm. There were 26 follicular adenomas with oncocytic features; the remainder had nodular hyperplasia. 21 cases (35%) had histologic lymphocytic thyroiditis (LT). 6 patients had simultaneous HC FNAs from different nodules; 4 were resected with nodular hyperplasia (1 also with LT). Of the 39 malignant nodules, 33 (85%) were multinodular by US with median size of targeted nodules of 2.3 cm. There were 15 follicular carcinomas (12 oncocytic/ type), 20 papillary carcinomas (14 follicular variants), and 4 poorly differentiated carcinomas. 13 cases (39%) had LT. There was no signifiant difference in malignancy rate for solitary or multiple nodules (P=0.79). Histologic presence or absence of LT (P=1.0) did not differ significantly between benign and malignant nodules nor did nodule size by US (P=0.49) or surgical resection (P=0.57).

Conclusions: Contrary to the notion that HC FNAs are more likely benign in the setting of nodular hyperplasia, we found no significant difference in malignancy rate of solitary or multiple nodules. Histologic LT and size did not differ between resected benign and malignant nodules. These findings suggest that the presence of multiple nodules does not warrant the use of the AUS category as an alternative to HUR in TBS.

379 Utility of Brachyury in Distinction of Chordoma from Cytomorphologic Mimics in Fine Needle Aspiration and Core Needle Biopsy

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Background: Chordoma is a neoplasm of notochordal differentiation that most often occurs in the axial skeleton. Separation from mimics such as chondrosarcoma and metastatic mucinous adenocarcinoma (ACA) is therapeutically important but diagnostically challenging, especially in limited biopsies. Immunohistochemistry (IHC) for T or brachyury, a nuclear transcription factor expressed in chordoma, has recently proven diagnostically useful in whole-tissue sections. Our aim is to compare brachyury with conventional markers (S-100, EMA, keratin) in distinguishing chordoma from mimics in fine needle aspiration (FNA) and core needle biopsy (CNB) samples.

Design: In total, 20 chordoma cases (8 FNAs and 12 CNBs), 10 FNAs of chondrosarcoma, and 12 FNAs of metastatic mucinous ACA were evaluated. IHC was performed on cell blocks and CNBs using a rabbit polyclonal antibody to brachyury (Santa Cruz). Nuclear staining in at least 5% of tumor cells was recorded as a positive result. IHC (S-100, EMA, pan-keratin, AE1/AE3) performed at the time of diagnosis was also reviewed.

Results: Of the 20 chordoma cases, 15 were axial skeleton primary tumors and 5 were metastases (spine, pelvis, lung). Brachyury was positive in 17 (85%) chordoma cases; 14 had multifocal nuclear staining of moderate to strong intensity and 3 had weak focal staining. There were 5 sets of concurrent FNA and CNB; 4 pairs were brachyury-positive in both samples, and 1 pair was brachyury-positive in the CNB and negative in the cell block. S-100, EMA, and keratin stains were available for 13 cases: 9 (69%) cases (including the 3 brachyury-negative cases) were positive for S-100 and keratin or EMA, and 4 cases were keratin-positive but S-100-negative. No nuclear brachyury staining was seen in chondrosarcoma or metastatic mucinous ACA, though 2 ACA cases showed cytoplasmic staining.

Conclusions: Brachyury separates chordoma from cytomorphologic mimics with high sensitivity and specificity in limited biopsies. As a single test, brachyury has higher sensitivity (85%) than a combined panel of S-100 and epithelial markers (69%). When added to the conventional panel, brachyury increases sensitivity to 100% without sacrificing specificity. Cytoplasmic staining in mucinous ACA is a potential pitfall when interpreting brachyury in samples with scant cellularity.

380 Differential Expression of Two Different TTF-1 and Napsin A Double Stain Antibodies: Utility in Detecting Lung Adenocarcinomas

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Background: Immunohistochemistry (IHC) for thyroid transcription factor -1 (TTF-1) and Napsin A are used to confirm the diagnosis of lung adenocarcinoma (ADC). This double stain has been shown to be useful in the diagnosis of ADC in cell blocks (CB) from fine needle aspirates (FNAs). An attempt to elucidate any statistical differences between the double staining patterns between comparable vendor antibodies (Leica and Dako) was addressed.

Design: 35 FNA cell blocks of lung ADC and 24 of lung squamous cell carcinoma (SqCCA),were selected. Double-staining IHC was performed using Leica and Dako antibodies for TTF-1 and Leica antibodies for Napsin A, with TTF-1 as a brown nuclear stain and Napsin A as a red cytoplasmic stain. The expression results were identified and compared. FISH assay was performed on SqCCAs with aberrant expression of TTF-1.

Results: Twenty six of 35 (74%) lung ADCs were positive for both TTF-1 and Napsin A using the Dako TTF-1 antibody. Two ADCs expressed TTF-1 only; 4 cases expressed Napsin A only. One of 23 SqCCAs was positive for both using Dako, with 1 additional SqCCA being positive for TTF-1 only. This differs significantly from the Leica staining results, in which 3 of 24 SqCCAs expressed double staining and 6 of 24 expressed TTF-1 only.

Staining Results

	ADC	SqCCA
Leica		
TTF-1/Napsin A	26/35 (74%)	3/24 (13%)
TTF-1 total positive	28/35 (80)	9/24 (38)
Napsin A total positive	29/35 (83)	3/24 (13)
Dako		
TTF-1/Napsin A	26/35 (74)	1/23 (4)
TTF-1 total positive	28/35 (80)	2/23 (7)
Napsin A total positive	30/35 (86)	1/23 (4)

Statistical Analysis

	Sensitivity	Specificity	PPV	NPV	Accuracy
Leica					
TTF-1/ Napsin A	74%	87	89	70	79
TTF-1 (total)	80	62	75	68	72
Napsin A (total)	82	87	90	77	84
Dako					
TTF-1/ Napsin A	74	96	96	71	83
TTF-1 (total)	80	91	93	75	84
Napsin A (total)	86	96	97	81	90

PPV, positive predictive value; NPV, negative predictive value

Two TTF-1 positive SqCCAs showed low level amplification by FISH assay not seen in the TTF-1 negative control SqCCAs. With Leica antibodies, 28 of 35 (80%) ADC were both positive, 2 were TTF-1 only positive, and 3 were Napsin A only positive.

Conclusions: The double IHC stain for TTF-1/Napsin A is useful in the diagnosis of lung ADC. The use of Dako TTF-1 yielded the better results, having the same sensitivity as Leica, but with a higher specificity. Dako antibodies express less staining of SqCCAs; this may be due to increased sensitivity of the Leica antibody, making it less useful in differentiating ADC from SqCCA.

381 Immunocytochemistry with p16INK4a (p16) and Ki-67 as Adjuncts to the Pap Test

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Background: Predicting which women with low-grade squamous intraepithelial lesions (LSIL) on cervical Pap tests have or will develop high-grade cervical intraepithelial neoplasia (CIN2+) on cervical biopsy remains problematic due to the poor specificity of the Pap test and the transient nature of most HPV infections. It has been reported that the specificity of the Pap test can be increased by the use of a dual immunocytochemical technique (dual stain) that tests for the co-expression ofp16 and Ki-67. We report our experience using this method on routine diagnostic material.

Design: Residual liquid based cytology cellular material from women with Pap tests interpreted as LSIL was collected between November 2010 and May 2011 and analyzed for p16 and Ki-67 expression using the dual stain. Cases were scored as positive if at least one dual-stained cell was present and negative otherwise, independent of cell morphology. Interpretation was performed by the three authors. Correlation with subsequent cervical histology was performed.

Results: The study set included 231 dual-stained Pap test slides (165 Surepath and 66 Thinprep) from women ranging in age from 17 to 62 (mean 30). Of these, 205 had correlative histology, with CIN 1 in 49.8%, CIN 2 in 10.7%, and CIN 3 in 7.8%. The dual stain was positive in 199 (86.1%) overall and in 175 (85.4%) of those with histologic correlation. Regarding cases with CIN1+ histology, the dual stain was positive in 126/140 (sensitivity 90.0%). For CIN2+ histology, the dual stain was positive in 37/38 (sensitivity 97.4%). Specificity was 24.6%for CIN1+ and 17.4% for CIN2+.

Conclusions: The dual stain can be performed in a routine histology laboratory with commonly used equipment and was easily interpretable independent of cellular morphology. Dual immunocytochemistry using p16 and Ki-67 on Pap test cells interpreted as LSIL yielded a high sensitivity for the detection of CIN2+ lesions on cervical histology, with specificity similar to that obtained with Pap test plus oncogenic HPV testing.

382 Frequency and Etiology of Unsatisfactory Cervical Cytology by ThinPrep® Method in a Tertiary Care Urban Setting – A Snapshot of Brief Duration

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Background: The rate of unsatisfactory cervical cytological specimens is usually higher (up to 3.5%) for ThinPrep® preparation (TP) as compared to the conventional or SurePath™ preparations.

Design: We studied 7,644 cervical cytology specimens (ages 13 to 84, mean- 38 years) over a brief period of 6 weeks for unsatisfactory TP preparations submitted to our tertiary care center with annual turnover of more than 80,000 cervical specimens. Out of these, 233 (3.1%) specimens were interpreted as unsatisfactory. We analyzed various etiologic factors responsible for unsatisfactory interpretation in these specimens.

Results: Several causes for inadequate interpretations were identified (Table 1).

Table 1. Causes associated with unsatisfactory interpretations.

Causes	Number of specimens (Total 233)	(%)
LUBE	100	43
LUBE & blood	40	17
LUBE & inflammation	9	4
LUBE, blood, & inflammation	2	1
LUBE & ACC	20	9
LUBE, ACC, & inflammation	1	1
ACC	4	2
ACC with inflammation (atrophic vaginitis)	4	2
Blood	4	2
Blood & Inflammation	4	2
Inflammation	7	3
Inflammation with scant squamous cells	1	1
Scant squamous cells	22	9
Acellular	13	5
Other (e.g. cell clumping without particular reason)	2	1

LUBE, lubricant-like material; ACC, Atrophic cellular changes.

The most frequent etiologic factor was association with lubricant-like material (LUBE) with or without additional components such as atrophic cellular changes (ACC), blood and/or inflammation. Five specimens reported previously as unsatisfactory with scant cellular component due to LUBE with ACC were repeated during this time period. All these five repeated specimens did not show LUBE in TP. Satisfactory cellularity was present with ACC in 3 of these repeated specimens. However, 2 specimens with ACC still showed scant cellularity with unsatisfactory interpretations.

Conclusions: Lubricant-like material (with or without blood) is the most common underlying cause of unsatisfactory TP. The next common association is ACC (with or without inflammation). Although, based on a small number during this brief period of observation, a few repeated cases achieved satisfactory cellularity after repeating the cervical cytology, which did not show LUBE in repeated specimens. However, the chance of unsatisfactory may persist in cases with ACC. A larger study over a longer duration with retrospective and prospective repeat findings is suggested and has been initiated.

383 Utility of Immunocytochemistry To Improve Sensitivity of BK Virus Detection in Urine of Renal Transplant Patients

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Background: BK virus (BKV), a human polyomavirus, has been recognized as a cause of severe kidney allograft dysfunction. Early diagnosis of BKV nephropathy is important because no specific antiviral therapy exists. The current treatment of BKV nephropathy (BKVN) consists of a reduction in immunosuppressant therapy. Urine cytology, detection of viral DNA in urine or blood, and renal biopsy are the main diagnostic tools. The purpose of this study was to determine whether immunocytochemical staining improves sensitivity for BKV detection compared to routine urine cytology.

Design: 50 urine specimens negative for BKV by routine screening were studied. 8 specimens positive for BKV by routine screening were used as positive controls. Thin prep methodology was used to prepare the slides. The slides were first stained with H&E then independently screened by three observers for BKV noting also any other findings such as atypia, degenerative changes and inflammation. Then, following removal of coverslips and rehydration, stained slides were tested for polyomavirus and evaluated by the same observers. Immunocytochemical identification of polyomavirus was performed using a rabbit polyclonal antibody and Envision+/AEC+ detection (Dako Corporation, Carpinteria, CA). The presence of any nuclear staining was considered as positive regardless of the number of stained cells.

Results: All 8 positive control cases were stained positive immunocytochemically. Of the 50 cases which were negative by routine examination, 8 cases (16%) showed positivity immunocytochemically. The positive cases were not specifically associated with atypia, degenerative changes or inflammation.

Conclusions: Urine cytology is an easy and rapid method of detecting decoy cells in renal transplant patients. However, routine screening does not detect all cases of BKV infection. IHC increases sensitivity of urine cytology screening for BKV by detecting cases with only rare infected cells. IHC should be considered in conjunction with routine screening, because early diagnosis of BKV is important in the management of renal transplant patients.

384 Diagnostic Utility of Endobronchial Ultrasound-Guided Fine-Needle Aspiration (EBUS-FNA): A Review of 593 Cases

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Background: Establishing a definitive and accurate diagnosis is important for the diagnosis of mediastinal lesions by endobronchial ultrasound-guided fine needle aspiration (EBUS-FNA) and can decrease the need for additional diagnostic work up. Our aim was to evaluate the diagnostic utility of EBUS-FNAs from our institution.

Design: A retrospective review of all EBUS-FNA biopsies procured at our institution over a three year period (2007 to 2010) was performed to look at the adequacy, cytological diagnosis, and the available histological follow-up. Cases were given an adequacy statement (satisfactory/SAT, less than optimal/LTO, or unsatisfactory/UNSAT) and a descriptive diagnosis at the time of final cytological diagnosis that was based on cytomorphology and in some cases ancillary study results. Indeterminate cases were those cases with a final diagnosis of atypical or suspicious.

Results: A total of 593 EBUS FNA specimens were obtained from 357 patients with a mean age of 61years (ranging from 23-90 years), including 420 SAT cases (71%), 107 LTO cases (18%) and 66 UNSAT cases (11%). Histological follow-up was available in 203 cases (34.2%).

Table 1: Cytological-Histological Correlation in 203 of 593 EBUS-FNA Biopsies

FNA adequacy & diagnosis	**Histology: Benign**	**Histology: Granulomas**	**Histology: Neoplasm**	**Histology: Malignant**	**TOTAL**
UNSAT	**17**	**6**	**0**	**7**	**30**
LTO	**32**	**12**	**0**	**11**	**55**
Negative	28	9	0	6	43
Granulomas	0	3	0	0	3
Atypical	4	0	0	4	8
Suspicious	0	0	0	1	1
SAT	**58**	**34**	**1**	**25**	**118**
Negative	45	11	0	4	60
Granulomas	0	21	0	0	21
Atypical	12	2	0	1	14
Suspicious	1	0	1	4	6
Positive	0	0	0	16	16
TOTAL	**107**	**52**	**1**	**43**	**203**

Malignancy was identified in 7 UNSAT (23%), 6 LTO negative (11%), and 4 SAT negative (3%) cases with a negative cytological diagnosis. The missed malignant tumors included 11 lymphomas (65%), 5 non-small cell carcinomas (29%), and 1 renal cell carcinoma (6%). There were no false positive diagnoses identified. The overall sensitivity and specificity were 80% and 100%, respectively. Of the 29 (14%) indeterminate cases, 10 (35%) were found to be malignant.

Conclusions: EBUS-FNA has a high specificity (100%) and sensitivity (80%) with few false negative diagnoses and no false positive diagnoses. Lymphomas were the most common cause for false negative results, followed by carcinomas. Ensuring adequate sampling is crucial in minimizing false negative diagnoses given that the percent of false negative diagnoses decreased from 23% in UNSAT cases to 3% in SAT cases.

385 The Role of HRHPV Reflex Testing in the Triage of Peri- and Post-Menopausal Women with LSIL Pap Tests

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Background: Current management recommendations for women with abnormal Pap tests (ASCCP, 2006) include a new option for the management of post-menopausal women with LSIL, consisting of reflex high-risk HPV (HRHPV) testing. Despite this recommendation, HRHPV testing for the triage of LSIL Pap tests is still not routinely used by many practitioners. The objective of this study was to evaluate the relationship between HRHPV results and follow-up diagnoses, particularly for women of peri- and post-menopausal status.

Design: At UNC Hospitals, women ≥40 years old with LSIL cervicovaginal cytology results from 2004 to 2008 were identified. Patients with HRHPV testing and subsequent cervicovaginal sampling results (cytology and/or biopsy; most severe diagnosis) within 2 years were reviewed. Follow-up diagnoses obtained were categorized into high grade (i.e., HSIL and ≥CIN2/VAIN2) and low grade pathology. Age was dichotomized at 50 to examine the impact of peri- and post-menopausal status. Fisher's Exact test and Mantel-Haenszel statistics were used to test the relationship of age, HRHPV testing and pathology group. Odds ratio (OR) was used to describe the measures of association.

Results: Of 164 evaluable women, median age was 46. Patients with HRHPV negative results had significantly lower odds of having high grade follow-up pathology (OR=0.14, p=0.003) relative to those who were HRHPV positive. The association remained significant after controlling for age (p=0.003). Women ≥50 years with negative HRHPV had a significantly lower percentage of high grade pathology (0% or 0/24) than those with positive HRHPV (21% or 9/43, p=0.02).

Table 1

	Age 40-49		**Age ≥50**	
	Low-Grade N (%)	**High-Grade N (%)**	**Low-Grade N (%)**	**High-Grade N (%)**
HRHPV Neg	28 (17)	2 (1)	24 (15)	0 (0)
HRHPV Pos	52 (32)	15 (9)	34 (21)	9 (5)

Conclusions: The current ASCCP guidelines for the management of LSIL Pap tests in post-menopausal women include three options: immediate colposcopy, repeat cytology at 6 and 12 months and reflex HRHPV testing. If the HRHPV is positive, colposcopy is recommended and if it is negative, repeat cytology at 12 months is recommended. Our study supports the use of reflex HRHPV testing for LSIL Pap tests in older women, especially those ≥50 years, and its routine use would decrease colposcopy referrals by almost 36%.

386 Validation of Cervista HPV16/18 in SurePath Pap Specimens Using a PCR-Based HPV Genotyping Assay

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Background: Cervista HPV 16/18 is a FDA approved HPV genotyping assay for detecting HPV16 and 18. However, validation studies of Cervista 16/18 in SurePath Pap specimen are scant. To evaluate validity of Cervista HPV16/18 in SurePath Pap specimens, we conducted a HPV genotyping study to verify the testing accuracy of Cervista HPV 16/18 using a PCR-based, commercially available HPV genotyping assay, EasyChip, in residual sample collected after routine Pap test at our cancer center.

Design: In Department of Pathology, we retrospectively selected 56 Cervista HPV positive specimens (SurePath vials with residual samples after completing routine Pap test) for the validation study. The specimens included 33 negative, 14 ASC-US, 7 LSIL and 2 HSIL Pap specimens. The patient's age ranged from 22-74 with a mean of 49

years. HPV genotyping was conducted using both Cervista HPV16/18 and PCR-based EasyChip HPV genotyping assay. The HPV genotyping results of Cervista HPV16/18 and EasyChip assay were compared.

Results: One case was disqualified based on the EasyChip internal control reading. Seven Pap specimens were tested negative by both Cervista HPV16/18 and EasyChip HPV assay. In the remaining 48 cases, Cervista HPV16/18 classified 16 positive and 32 negative cases. Of 16 Cervista HPV 16/18 positive cases, 15 were confirmed to be either HPV16 or HPV18. One specimen classified HPV16+ by Cervista HPV 16/18 showed non-HPV16 HPV types by EasyChip assay. Of 32 Cervista HPV16/18 negative specimens, 30 were confirmed to have non-16/18 HPV types by EasyChip assay. HPV18 was detected in 2 Cervista 16/18 negative specimens. The concordance between Cervista HPV16/18 and EasyChip HPV genotyping assay is 94%. Discrepancies between the two HPV genotyping assays occurred in Pap specimens with either negative (2 cases) or ASC-US (1 case) results.

Conclusions: Our HPV genotyping results support the validation of Cervista HPV16/18 as a reliable HPV16/18 genotyping assay in SurePath Pap specimens. Testing discrepancies between Cervista HPV 16/18 and EasyChip HPV genotyping assay were observed in a small fraction of specimens. Further study is required to clarify the issue. Table1.

HPV Genotyping Agreement between Cervista HPV16/18 and EasyChip HPV Assay in SurePath Pap Specimens (n=48)

Pap	A9/HPV16	A7/HPV18	A9/other type	A7/other type	A5/6	Not match
Negative	2	1	9	7	5	2
ASC-US	4	1	3	3	2	1
LSIL	4	1	1	0	0	0
HSIL	2	0	0	0	0	0
Total	12	3	13	10	7	3

387 Validation of EGFR Testing on FNA Cytology and Core Biopsy Samples on the Qiagen Rotor-Gene System

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Background: Epidermal growth factor receptor (EGFR) mutation detection in pulmonary adenocarcinoma is of increasing importance for determination of appropriate EGFR directed therapy. If valid results can be obtained on small biopsy or fine needle aspiration samples, it will be extremely valuable for further treatment. Few comparative studies have been performed on different sample types. This study presents an optimization for FNA and surgical samples on the Qiagen Rotor-Gene and the PyroMark Q24 platforms and to compare the results on both FNA and surgical specimens.

Design: Surgical specimens and cytology cell blocks were used as formalin fixed tissue (FFPE) and compared to cytology smears obtained from FNA and pleural fluids. Laser capture microscopy was used to enrich for tumor in FFPE and cell block samples and DNA was extracted. Semiquantitative measurement of mutations in exons 18-21 on the PyroMark Q24 was compared with the qualitative measurement of mutations in exons 18-21 on the Rotor-Gene instrument which utilizes ARMS and Scorpions technology.

Results: 54 formalin fixed biopsy, lung resection specimens, FNA and cytology cell blocks (FFPE) and 43 air dried direct smears were used. This data was collected from 90 patients, 16 of who had matched cytology and surgical pathology specimens. DNA extracted from smears ranged from 0.11-244.18 ng/ul and from FFPE samples ranged from 5.22-127 ng/ul. Number of cell groups on the cytology samples ranged from 1-15. The PyroMark Q24 which has a high specificity and sensitivity with 5% mutation cut-off limit was used as the standard against which the Rotor-Gene results were compared.

Sensitivity and Specificity on the RotorGene

CYTOLOGY SMEARS	PyroMark Q24	
Rotor-Gene	Positive	Negative
Positive	2	1
Negative	4	36
	Sensitivity=33%	Specificity=97%
FFPE	PyroMark Q24	
Rotor-Gene	Positive	Negative
Positive	8	1
Negative	7	32
	Sensitivity=47%	Specificity=97%
Invalid (9)		

Results on air dried smears were compared to FFPE (using PyroMark Q24 results only). 6/43 cases of cytology and 17/54 cases of FFPE were positive. 15/16 matched smear and FFPE cases showed identical results.

Conclusions: Direct extraction and analysis of EGFR mutations from cytology smears is a convenient and robust method for FNA obtained samples. This validation allows for collection of additional smears on site during FNA procedures, and substantially decreases the time it would take to perform the testing on the final resection specimen. Based on our preliminary data, the PyroMark Q24 platform is more sensitive than the Rotor-Gene platform.

388 Computed Tomography-Guided Fine Needle Aspiration and Needle Core Biopsy: Which Specimen Type Yields Diagnostic Results?

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Background: Computed tomography (CT) guided sampling of deep seated lesions throughout the body has become a standard diagnostic procedure. Sampling methods of such lesions include both fine needle aspiration (FNA) cytology and needle core biopsy (NCB). Previous studies have shown contradictory results in sensitivity and specificity of these modalities, and few studies have compared these procedures among different interventional radiologists. The aim of this study is to compare the diagnostic success rates of CT-guided FNA versus NCB at our institution.

Design: We retrospectively reviewed a total of 141 patient samples from January 2008 to December 2009 in which both FNA and NCB material was available. Sample procurement was performed by five interventional radiologists. These consisted of lung (105), liver (16), and lymph node (20) cases. Unsatisfactory FNA cases were excluded. FNA samples underwent immediate assessment and follow-up evaluation by a cytopathologist. Those obtained by NCB underwent histologic evaluation. The final diagnosis was confirmed by surgical resection or clinical findings.

Results: FNA cytology identified 100 of 109 (92%) malignant lesions with a false negative rate of 9%. NCB histology detected 87 of 109 (80%) malignant lesions with a false negative rate of 25%. Twenty-six cases had corresponding benign cytology and histology. Six cases were unsatisfactory by FNA; of those, 2 were benign and 4 were malignant by NCB. There was 72% correlation rate between FNA and CNB. More accurate tumor classification was possible in 4 cases on NCB. Only minor variations were noted based on which interventional radiologist was performing the procedure.

		NCB		Site
		Positive	Negative	
FNA	Positive	63	19	Lung
		6	3	Liver
		9	0	Lymph Node
		78	22	Total
	Negative	6	14	Lung
		1	5	Liver
		2	7	Lymph Node
		9	26	Total

Conclusions: Both CT-guided FNA and NCB can serve as sampling methods of deep seated lesions. While FNA allows for immediate assessment and biopsy guidance, NCB can result in more abundant tissue for complete histologic evaluation. In our study, FNA had a higher malignancy detection rate with a lower false negative rate when compared to NCB. This is likely due to the use of immediate assessment at our institution. Thus, the role of FNA cytology is an important diagnostic tool; however, utilization of each modality ultimately depends on the proper clinical setting and the experience of the interventional radiologist and pathologist.

389 Utility of Immunocytochemistry on Direct Smear Preparations in the Diagnosis of Effusions

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Background: Metastatic malignancy represents a common cause of effusions. Immunocytochemistry (ICC) is useful in confirming a diagnosis of malignancy and gaining insights into sites of origin. Cell blocks are commonly utilized for this purpose; nonetheless, it is not uncommon for the malignant cells to present as a minority population within the milieu of background mesothelial cells and histiocytes. In cases where the malignant cells are sparse, they may not be represented in cell blocks thereby precluding immunophenotypic characterization. Thus, we sought to investigate the utility of direct smear preparations as a platform for ICC in the diagnosis of effusions.

Design: Air-dried, unstained direct smears were prepared for 48 malignant effusions and 17 reactive effusions for comparison. ICC for the following markers was performed following brief formalin fixation and antigen retrieval: EMA, MOC-31, p63, TTF-1, Napsin-A, PAX8, CDX-2, and calretinin.

Results: The results of ICC for the malignant effusions are summarized in the table below.

ICC Results

Malignancy (# cases)	Immunostain	# Positive (%)
Mullerian ADC (n=15)	EMA	15 (100%)
	MOC-31	15 (100%)
	PAX8	15 (100%)
Lung ADC (n=9)	EMA	9 (100%)
	MOC-31	9 (100%)
	Napsin-A	7 (78%)
	TTF-1	6 (67%)
Pancreatic ADC (n=5)	EMA	3 (60%)
	MOC-31	5 (100%)
	CDX-2	3 (60%)
Gastric ADC (n=4)	EMA	3 (75%)
	MOC-31	4 (100%)
	CDX-2	1 (25%)
Breast ADC (n=4)	EMA	4 (100%)
	MOC-31	4 (100%)
Urothelial carcinoma (n=4)	p63	3 (75%)
Mesothelioma (n=2)	EMA	2 (100%)
	MOC-31	1 (50%)
	Calretinin	2 (100%)
Colorectal ADC (n=2)	EMA	1 (50%)
	MOC-31	2 (100%)
	CDX-2	2 (100%)
Lung squamous cell carcinoma (n=1)	p63	1 (100%)
	Napsin-A	0 (0%)
	TTF-1	0 (0%)
Papillary thyroid carcinoma (n=1)	EMA	1 (100%)
	MOC-31	1 (100%)
	PAX8	1 (100%)
	TTF-1	1 (100%)
Anaplastic thyroid carcinoma (n=1)	EMA	1 (100%)
	MOC-31	1 (100%)
	PAX8	1 (100%)
	TTF-1	0 (0%)

Abbreviations: ADC, adenocarcinoma

Overall, EMA and MOC-31 immunoreactivity was observed in the tumor cells in 91% and 98% of malignant effusions, respectively. EMA immunoreactivity was focally observed within the calretinin(+) mesothelial cell population in 2 (12%) of 17 reactive effusions. Immunostains for MOC-31, p63, TTF-1, Napsin-A, PAX8, and CDX-2 were negative in all 17 reactive effusions.

Conclusions: Direct smears represent an effective platform for the performance of ICC in the diagnosis of effusions. ICC for calretinin, p63, TTF-1 & Napsin-A, PAX8, and CDX-2 are especially helpful for confirming metastases from mesothelioma, urothelial cell carcinoma & squamous cell carcinoma, lung adenocarcinoma, Müllerian adenocarcinoma, and gastrointestinal/pancreaticobiliary adenocarcinoma, respectively.

390 High-Risk Human Papilloma Virus (hrHPV) Positivity Rates with Histologic Correlation in Postmenopausal Women with Low Grade Squamous Intraepithelial Lesion (LSIL)

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Background: There are several challenges inherent to the interpretation of the Papanicolaou (Pap) test in postmenopausal women, posing difficult management issues. The ASCCP acknowledges that reflex testing for hrHPV in postmenopausal women with LSIL is an acceptable option for colposcopic triage. However, there is limited data available regarding the follow-up of such patients based on their hrHPV results.

Design: A retrospective database search was conducted for the time period January 2008 to June 2011 for all cervical Pap tests (ThinPrep) with a diagnosis of LSIL or LSIL, high grade cannot be excluded (LSIL-H) in women ≥ 50 years of age (considered postmenopausal for the purposes of this study). The results of hrHPV co-testing (Hybrid Capture 2), if available, and histologic follow-up including colposcopic biopsy and/or endocervical curettage within the next 6 months, if any, were compiled. The two groups were compared using the chi-square test and a p value of < 0.05 was considered to be statistically significant.

Results: 384 patients were identified with LSIL during the study period, of which 246 were co-tested for hrHPV. Amongst these, 191 (78%) patients were hrHPV positive. 224 patients had histologic follow-up out of which 14 (6%) patients had CIN2 or above. LSIL-H was detected in 68 patients - 48 of these were co-tested for hrHPV out of which 44 (92%) were positive and 55 of these had histologic follow-up out of which 21 (38%) had CIN2 or above. None of the patients with a negative hrHPV result in either group were associated with CIN2 or above on follow-up. The differences between the rates of hrHPV detection and of subsequent high grade dysplasia on histologic follow-up in the LSIL and the LSIL-H groups were found to be statistically significant.

Conclusions: Our study reveals a high rate of hrHPV positivity (approximating the detection rate of the ASCUS/LSIL triage study of 82.9%) but a low prevalence rate of high grade squamous dysplasia in our postmenopausal patients with LSIL. The LSIL-H patients exhibited an even higher rate of hrHPV positivity and a much higher prevalence of high grade squamous dysplasia. These results indicate that reflex hrHPV testing has little potential value in LSIL-H patients and also question its utility in LSIL patients in this age group. Additional data that is more representative is needed to determine the most optimal option to guide clinical decision-making in this cohort.

391 Usefulness of Cytological Samples for the Assessment of ALK Gene Rearrangements in NSCLC Patients

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Background: ALK gene rearrangement defines a new molecular subtype of NSCLC. Crizotinib, a dual MET and ALK inhibitor, shows promising efficacy in these patients. To date, determination of EML4-ALK fusions has been performed in biopsies or surgical specimens. However, advanced lung cancer is often diagnosed in cytology specimens obtained through fine needle aspiration (FNA), and frequently cytological specimens are the only tumor samples available. We evaluated the possibility of determining ALK gene rearrangements in cytological samples from NSCLC patients.

Design: Between January and September 2011, 42 cytological samples from 42 NSCLC patients (25 M and 17 F) were analyzed for ALK gene rearrangements by FISH (Vysis dual colour break apart probe). Tumour samples were obtained by bronchoscopy guided FNA (21 cases-50%), EBUS-FNA (2 cases-4.8%), EUS-FNA (7 cases-16.7%), CT-FNA (2 cases-4.8%), and direct FNA (2cases- 4.8%). We also studied 2 cavity fluids (4.8%), 4 imprints from surgical specimens (9.5%), and 2 cellblocks received for consultation. FISH analysis was performed on Papanicolau stained smear in 15 cases (35.7%), non-stained ThinPrep in 21 cases (50%), cell block in 4 cases (9.5%), and 1 in a stained ThinPrep. All cases were previously tested for EGFR and KRAS mutations.

Results: Twenty-nine samples (69%) were adequate for FISH analysis. Two cases were positive (6.9%), both women and non-smokers of 36 and 73 yo having adenocarcinoma with signet ring cells. FISH analysis was done on ThinPrep unstained slides. One case had a concurrent EGFR mutation in exon 21. FISH study was unsuccessful in 13 cases. Ten of them were performed on Papanicolau stained smears (76.9%), and 3 on unstained ThinPrep (14.3%). All paraffin embedded samples, and 19 (86.4%) TrinpPrep were adequate for FISH analysis. Correlation between cytological and surgical samples has been performed so far in 4 cases, with a concordance rate of 100%.

Conclusions: Determination of ALK gene rearrangements in cytological specimens is feasible. ThinPrep and cell blocks are the most suitable samples for FISH analysis, while Papanicolau stained smears provide poor results. Coexistence of ALK gene rearrangement and EGFR mutation, although rare, are not mutually exclusive. Analysis by FISH of ALK gene rearrangement on ThinPrep slides could be an option when no paraffin embedded tissue is available.

392 Utility of ProExC and IMP3 Immunocytochemical Staining of Atypical Glandular Cells of Undetermined Significance (AGUS) in Liquid-Based Cervical Cytology

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Background: Studies have reported positive ProExC and IMP3 staining in neoplastic glandular lesions of the uterine cervix and corpus. Thirty percent of cases diagnosed as AGUS in liquid-based cervical cytology specimens have significant underlying pathology, while the remaining are related to reactive, reparative and metaplastic conditions. We address the utility of these markers in the evaluation of AGUS cases in liquid-based cervical cytology.

Design: Unstained Thin-Prep slides were prospectively collected on all cases diagnosed as AGUS from June 2007 to November 2009. Based on the follow-up biopsy findings in thirty-four cases (n=34), the study included cases of adenocarcinoma in situ (AIS, n=2), adenocarcinoma (AC, n=3), squamous cell carcinoma (SCC, n=1), LSIL (n=3), HSIL (n=4) and benign (n=21). ProExC (TriPath Imaging, prediluted) and IMP3 (Dako, dilution 1:100) immunocytochemical (ICC) stains were performed. The results were correlated with subsequent biopsy findings. Positivity was assessed by strong diffuse nuclear (ProExC) and granular cytoplasmic (IMP3) staining.

Results: The presence of AGUS cells on the ICC stained slides was confirmed in all cases. Of all glandular lesions, IMP3 was positive in 4/5 cases and negative in 27/29 non-glandular lesions/benign cases (sensitivity: 80%, specificity: 93%, negative predictive value - NPV: 96%); ProExC was positive in 3/5 glandular lesions and negative in 24/29 non-glandular lesions/benign cases (sensitivity: 60%, specificity: 82%, NPV: 96%). When used as a panel (ProExC + IMP3), at least one stain was positive in 5/5 glandular lesion cases and they were both negative in 24/29 non-glandular lesions/benign cases (sensitivity: 100%, specificity: 83%, NPV: 100%). Of the 5 cases of non-glandular lesions/benign cases in which at least one was positive, the follow-up diagnosis was SCC (n=1), HSIL (n=2), LSIL (n=1) and benign (n=1).

Conclusions: ICC staining for ProExC and IMP3, particularly when used as a panel, might serve as a predictor of glandular lesions on subsequent biopsies, as our study demonstrated positivity of at least one of the two markers in all AIS and AC lesions. Positivity also predicted the presence of squamous lesions in subsequent biopsies. Our findings suggest a role for IMP3 and ProExC ICC staining as an aid in the characterization and potential management of AGUS cases.

393 Melamed-Wolinska Bodies Are an Additional Cytological Feature of Metastatic Urothelial Carcinoma

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Background: Melamed-Wolinska bodies (MWB) are round and pink intracytoplasmic inclusions of unknown significance found within degenerating benign or malignant urothelial cells in Pap-stained urine specimens. MWB have previously been described in pleural fluid specimens with metastatic urothelial carcinoma (MUCa) (*Acta Cytol.1997;41:995*). We investigated the presence of MWB in fine needle aspiration (FNA) specimens of MUCa and studied their utility *vis a vis* other established MUCa cytologic characteristics.

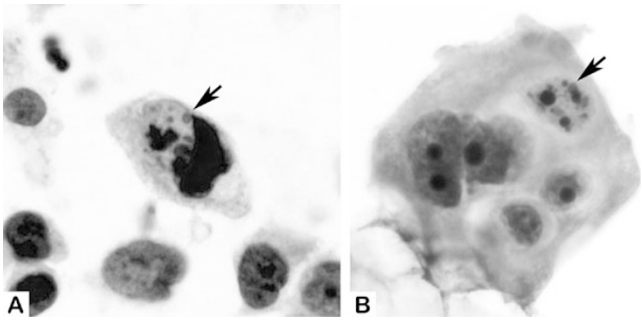
Design: Our database was searched for all cases of MUCa diagnosed over a 21-month-period (01/2010–09/2011). In addition, carcinomas (ca) of other primary sites including colonic, 3; hepatocellular, 2; pancreatic, 6; pulmonary adenoca, 10; and pulmonary squamous cell ca, 4 were obtained as control. All Pap-stained slides (Direct Smear, ThinPrep, and Cytospin) for each case were reviewed by two pathologists for MWB and other known features of MUCa: cercariform cells, pearl formation, and cannibalism.

Results: Twenty-one cases of MUCa were studied; 9 women and 12 men with ages ranging from 57 to 90 (mean-75). In MUCa cases, 76% had cercariform cells, 67% had identifiable MWB, 24% had cannibalism, and 19% had pearl formation. MWB were present in viable and degenerating tumor cells in MUCa cases and were seen in all three forms of preparation. In 25 control cases, the frequency of these features was as follows: cannibalism, 20%; cercariform cells, 12%; MWB, 12% and pearl formation, 4%.

Cytological Features in Metastatic Urothelial Carcinoma and Carcinoma of Other Primary Sites

Origin	# of cases	Cercariform cells	Pearls	Cannibalism	MWB
Urothelial	21	16	4	5	14
Colon	3	0	0	2	0
Liver	2	0	0	0	0
Pancreas	6	0	0	1	1
Lung-Adeno	10	3	1	2	1
Lung-SCC	4	0	0	0	1

Figure 1:MWB (arrows) in a Pap-stained direct smear (A) and ThinPrep (B) from two cases of MUCa.



Conclusions: In this series (1) MWB were present in two-thirds (67%) of MUCa cases. (2) In decreasing frequency, cercariform cells, MWB, cannibalism, and pearl formation were identified in MUCa. (3) While not unique to MUCa, they may be considered an additional cytological feature for this diagnosis.

394 Histologic Follow-Up Results in Patients with Pap Test Findings of Endometrial Cells: Results from a Large Academic Women Hospital Laboratory

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Background: TBS 2001 recommendation to report normal endometrial cells (nEMC) in women ages ≥40 years. nEMC in women ages ≥40 years and atypical endometrial cells (aEMC) of Pap remain challenges for interpretation and management. We reported the largest histopathologic follow-up findings for women with endometrial cells (both nEMC and aEMC) in Pap testing.

Design: We searched our pathology databases (a large academic medical center) from 2005 to 2011 to retrieve cases with Pap tests of nEMC in women ages ≥40 years, aEMC or endometrial carcinoma cells (EMCC). Pap tests were liquid-based cytology (LBC) specimens screened using computer-assisted screening. Follow-up diagnoses were correlated with cytology and stratified into age groups.

Results: During a period of 6 years, 1,183 cases with a cytology report of endometrial cells (nEMC, aEMC and EMCC) were documented with histopathologic follow-up. Significant endometrial lesions (precancerous and malignant) were found in women with aEMC (18.3%) or with EMCC (100%) Pap testing (Table 1).

1183 patients with a Pap testing of endometrial cells and histologic follow-up

	Negative (%)	Benign (%)	Precancerous (%)	Malignant (%)	Total	p value
nEMC	532 (72%)	187 (25%)	13 (1.8%)	7 (0.95%)	739 (100%)	
aEMC	227 (54%)	118 (28%)	18 (4.3%)	60 (14%)	423 (100%)	<0.01
EMCC	0	0	0	21 (100%)	21 (100%)	<0.01
Total	759 (64%)	305 (26%)	31 (26%)	88 (7.4%)	1183 (100%)	

More importantly, significant endometrial lesions were also found in women ≥50 years with nEMC after Day 12 of menstrual period (5.19%). However, no significant endometrial lesions were found in women with nEMC either before Day 12 (0.51%) or women younger than 50 years after Day 12 (1.58%) (Table 2).

Histologic follow-up in 739 patients with nEMC

	Precancerous/malignant lesion # (%)	Total case #	p value
nEMC ≤12 d	1 (0.51)	197	
nEMC >12 d, <50 yr	4 (1.58)	253	0.16
nEMC >12 d, ≥50 yr	15 (5.19)	289	<0.01
total nEMC >12 d	19 (3.51)	542	<0.01

Conclusions: Our data indicated that endometrial sampling has no clinical benefit in women (regardless of ages) with a nEMC before Day 12 of menstrual cycle or women younger than 50 years with a nEMC after Day 12. Endometrial sampling should be routinely performed in women with aEMC and women older than 50 years with nEMC.

395 Nuclear Pseudo-Inclusions Are Rare, but Nuclear Grooves Are Nearly Always Present, in Cytopathology and Histopathology Material of Encapsulated Follicular Variant of Papillary Thyroid Carcinoma

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Background: Encapsulated Follicular Variant of Papillary Thyroid Carcinoma (EFV-PTC) can be diagnostically challenging in cytopathology as well as histopathology material; mainly because its nuclear features can often be focal and subtle; and remain, thus far, a diagnostic challenge.

Design: All archived histopathology slides from surgically-resected EFV-PTC cases (2006-2011) were retrieved. Diagnosis was confirmed *(Mod Pathol 2011;24:S1-S9, Acta Cytol 2002;46:555-561)*. Corresponding fine needle aspiration (FNA) cytopathology preparations (Diff-Quik and/or Pap) were retrieved. Consensus review (2 observers) was undertaken of all histopathology and cytopathology material, particularly with regards to nuclear features.

Results: Table 1 shows the results of the nuclear features based on the aforementioned review.

Nuclear Features of EFV-PTC Cells in Cytopathology and Histopathology

	Cytopathology	Cytopathology Histopathology
Grooves	19/20 (95%)	20/20 (100%) *
Elongation	19/20 (95%)	20/20 (100%)
Overlap	19/20 (95%)	20/20 (100%)
Ground Glass	18/20 (90%) **	20/20 (100%)
Irregular Shape	17/20 (85%)	20/20 (100%)
Pseudo-inclusions	02/20 (10%)	01/20 (5%) ***

Note: Most features (except grooves, vide infra) were readily identifiable in most preparations. *: Nuclear grooves were diffusely present in 15/20 and only focally present on histopathology material in 5/20 (25%) cases. **: In general, Pap and Diff-Quik preparations were equivalent and similar with regards to nuclear findings, except for evaluation of "ground glass" nuclei (inherently problematic on Diff-Quik). Ground glass nuclei were also a less prominent feature in histopathology material ***: The only case of EFV-PTC which showed nuclear pseudo-inclusions in histopathology material displayed solid features of PTC as well. Data on control cases, and other pathological features (including cellularity, characteristics of follicles and colloid, etc.) were non-contributory and are not shown herein.

Conclusions: Based on this series of EFV-PTC cases, nuclear pseudo-inclusions are a rare finding in cytopathology (10%) and histopathology (5%) preparations; however, nuclear grooves are evident in 100% of cases (but this finding may be focal in histopathology material in a minority of cases).

396 The Emerging Technique of Electromagnetic Navigation Bronchoscopy-Guided FNA of Peripheral Lung Lesions: Promising Results in 51 Patients

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Background: Electromagnetic Navigation Bronchoscopy [(ENB), *superDimension, Inc., Minneapolis, MN*], is an emerging "GPS-like" system of bronchoscope used for pathologic evaluation of peripheral lung lesions [(PLL), outer 1/3 of chest] not visualized by standard bronchoscopy. Diagnostic yield of bronchoscopy for <2cm PLL is 14% (*Am J Respir Crit Care Med. 2006;174:982*). Compared to CT, ENB has less radiation and complications. Literature on fine needle aspiration (FNA) performed by ENB technique is scarce. Herein, we report our experience with ENB-FNA.

Design: Cases of PLL sampled by ENB at 2 institutions over 51-months (07/'07 to 09/'11) were reviewed. Details of ENB technique have been published (*Ann Thorac Surg 2008;85:S797*). Briefly, all patients had a thin-slice non-contrast CT to create a 3-dimensional re-construction of the bronchial tree and the PLL was marked. ENB was then used to guide bronchoscopic biopsy (BX) tools to the PLL. A FNA with rapid on-site cytologic evaluation (ROSE) was performed in all cases.

Results: 51 patients (pts) (M=42; F=9; age range: 49 to 88, mean: 66) were studied. 11/51 had a history of non-pulmonary malignancy. All cases had ROSE. PLL size ranged from 0.3cm-7.0cm (mean: 2.5cm). Overall, diagnostic tissue was obtained in 39/51 (76%) pts on ENB-FNA. Diagnostic yield of ENB-FNA was not significantly different by lesion sizes (73% for <2cm *vs.* 88% for >4cm, *p-value=0.245*). FNA diagnoses were: Malignant in 25 (24 primary and 1 metastatic); Benign/inflammatory (NEG), 19; Non-Diagnostic (ND), 7. The 24 primary cases included: adenocarcinoma (ACa), 15; squamous cell Ca, 4; non-small cell Ca, 3 and small cell Ca, 2. The 1 metastasis was urothelial Ca. 10/51 (20%) were false-negative (5 cases each from the NEG and ND category) and were considered ENB sampling error based on histologic follow-up. Sensitivity and specificity of ENB-FNA was 68% and 100%. Average time spent by the cytopathologist on-site was 45 min. Pneumothorax occurred in one pt.

Conclusions: In this series, evaluation of PLL by ENB-FNA has an overall sensitivity of 68% and specificity of 100% which is equivalent to other published series of ENB-FNA of PLL. Diagnostic yield for PLL <2cm is better compared to bronchoscopy and complication rate is lower compared to CT-guided FNA.

397 Fine Needle Aspiration Biopsy of Palpable Breast Masses Is Associated with Shorter Length of Time to First Treatment Compared with Core Biopsy

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Background: Time to treatment has been shown to be an important measure of quality in cancer care. Fine Needle Aspiration Biopsy (FNAB) for the evaluation of breast lesions remains underutilized and has been supplanted by core biopsy (CB) in many centers. We compared time to treatment in two matched patient cohorts with palpable breast masses diagnosed by either FNAB or CB.

Design: We reviewed medical records of all patients who received FNAB or CB in 2009 for palpable breast masses at two affiliated tertiary academic medical centers. One center utilized a pathologist-run FNAB clinic and the other utilized CB almost exclusively. Data retrieved from the common lab information system included age, lesion size (by palpation for FNAB, by imaging for CB), diagnosis, and time from presentation to excision or neoadjuvant therapy (NEO).

Results: Of 257 total FNAB, 51 went to excision and 3 received NEO (mean age 52; range 19-92). Of 1346 total CB, 103 were excised and 15 were treated with NEO (mean age 52; range 18-87). At time of biopsy, median breast mass size for FNAB was 1.5 cm (range 0.4-7.0) and for CB was 1.7 cm (range 0.3-6.0). Median carcinoma size for FNAB was 1.8 cm (mean 2.09, range 0.9-3.1) and for CB was 2.1 cm (mean 2.35, range 0.4-8.0). Median time from presentation to excision or NEO was shorter for FNAB patients.

Length of Time to Treatment (days)

	All Patients	All Patients	All Patients	Benign Lesions	Benign Lesions	Benign Lesions
	Mean	Median	Range	Mean	Median	Range
FNAB	67.02	38.5	2-294	108.5	87.5	9.0-294
CB	58.44	44	9-402	175.17	155.0	56-402

Length of Time to Treatment (days)

	Indeterminate Lesions	Indeterminate Lesions	Indeterminate Lesions	Malignant Lesions	Malignant Lesions	Malignant Lesions
	Mean	Median	Range	Mean	Median	Range
FNAB	69.8	44	11-288	34.16	32.0	2.0-66
CB	59.81	51.0	20-180	49.61	41.0	9.0-239

Conclusions: Patient age and lesion size were similar for FNAB and CB cohorts at time of biopsy. Median size of carcinoma at excision was slightly smaller for FNAB cases. Median length of time from presentation to first treatment was shorter for patients diagnosed by FNAB compared with CB in all diagnostic categories. These findings suggest FNAB is advantageous for faster triage of breast patients to treatment. Careful selection of patients with palpable breast lesions appropriate for FNAB evaluation may improve patient care.

398 Endobronchial Ultrasound-Guided Transbronchial Needle Aspiration (EBUS): The Impact on the Cytology Laboratory

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Background: Endobronchial Ultrasound-Guided Transbronchial Needle Aspiration (EBUS) plays an important role in the staging of lung carcinoma and allows sampling of intrathoracic lymph nodes. EBUS has great impact on any Cytology laboratory. All slides, including cell block slides, are reviewed in the laboratory, each requiring approximately 5 minutes for review. This overall process consumes hours of cytotechnologist time. Time spent away from the laboratory to assist in such procedures limits the time left in the day for cytotechnologists to screen slides. Also, strict federal and state laws limit the number of slides that a cytotechnologist may see in any 24 hour period to 100, a number that is prorated for an 8 hour work day. In such circumstances, the laboratory risks exhausting the legal capacity of the cytotechnologists' screening time. Although time consuming to the laboratory, EBUS offers advantages to patients when compared to mediastinoscopy. Our objective is to retrospectively study all the slides made at each procedure and identify any trends in sampling that may lead to more efficiency in the performance of this procedure.

Design: 203 cases from 92 patients who had EBUS lymph node sampling, collected from March 2009 through August 2011 in Danbury Hospital, were retrospectively reviewed in their entirety. Cases include direct smears, ThinPrep slides and cell block slides.

Results: Of all the cases, 2238 total slides were made with an average of 11 slides per case (range 3-39).

	No. Of cases (sites)	Total no. Of slides	Average no. Of slides per site (range)	Cytotech time screening per site (minutes)
Diagnostic cases	49	536	11 (3-25)	55
Non-diagnostic cases	153	1702	11.12 (3-39)	55.6

* One case of Hodgkin lymphoma is excluded

At least two different lymph node sites were sampled per patient. The average total time consumed by cytotechnologists, including their time spent in the endoscopy suite (75 min per cytotech) per patient is approximately, on average 260 minutes (4.3 hours).

Conclusions: EBUS offers advantages to patients, however, the impact on the Cytology lab is significant, particularly in view of the strict federal laws limiting the workload of cytotechnologists. The value of this time-consuming and labor-intensive technique is not reflected in measures of the laboratory's productivity or RVUs (relative value units). Review of all slides revealed that the ThinPrep and cell block in the vast majority of cases contained diagnostic cells, suggesting that there may not be a need to prepare as many smears as is currently done.

399 A Single Institution Experience with the New Bethesda System for Reporting Thyroid Cytopathology: Correlation with Existing Cytologic, Clinical and Histological Data

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Background: The Bethesda system for reporting thyroid cytopathology (TBS) is a 6-tiered diagnostic system with specific recommendations for follow-up based on malignancy risk. The goal of this study was to compare it to the previously used classification system at our institution.

Design: 100 consecutive thyroid FNAs collected in 2006 were independently reviewed by two cytopathology-boarded pathologists. Diagnosis was based on TBS. Cases with discordant diagnoses were reviewed in a consensus conference for final diagnosis. Cases in which a final consensus could not be reached were excluded. Consensus diagnoses were compared to 2006 diagnoses, and were correlated with histologic and clinical follow-up.

Results: 97 cases were included in the study. Table 1 shows the distribution of cytology diagnoses. Interobserver agreement across all TBS categories was 0.4778. 28 cases had follow-up histology. Of these, 16 were benign and 12 were malignant. Of the cases called benign in 2006, 22 were called unsatisfactory in 2011. Cases were more likely to be unsatisfactory if there were < 3 passes or if there was no on-site pathology evaluation. Cases called follicular lesion in 2006 had both benign and malignant histology (12.5% malignant, 87.5% benign, n = 8). Clinical follow-up of these cases included repeat FNA (21%), resection (57%), imaging (36%) and none (21%). In contrast, all cases called follicular neoplasm in 2011 had actionable diagnoses by histology. The positive predictive value was 1.0 for actionable diagnoses in 2006 and 2011. The negative predictive value for an actionable diagnosis was 0.533 in 2006 and 2011.

Distribution of Cytology Diagnoses

	2006	2011
unsatisfactory	15.5%	38.1%
benign (NAMC)	59.8%	42.3%
AUS (atypia)	4.1%	7.2%
follicular lesion	14.4%	n/a
follicular neoplasm	1.0%	7.2%
suspicious for malignancy	2.1%	0
malignant	3.1%	5.2%

Conclusions: Using strict TBS criteria for a satisfactory specimen increased the unsatisfactory rate. Fewer passes and lack of involvement of pathology in the procurement, preparation, and screening of slides led to increased unsatisfactory specimen. The inter-observer variability was in moderate agreement (kappa = 0.4778) using the Bethesda criteria. The pre-Bethesda diagnosis of follicular lesion led to inconsistent clinical management. More clarification is anticipated in clinical management for specific cytopathologic diagnostic categories using TBS.

400 Arginase-1: A Novel Immunohistochemical (IHC) Marker of Hepatocellular Differentiation in Fine Needle Aspiration (FNA) Cytology

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Background: FNA biopsy under radiologic guidance is a safe and effective method of assessing lesions in the liver. Unfortunately, diagnostic pitfalls exist in the distinction of hepatocellular carcinoma (HCC) from other primary and metastatic mass lesions in liver FNA specimens as a result of limited sample size, the absence of full architectural detail, and the presence of significant cytomorphologic overlap. Arginase-1 (AG-1) is a urea cycle enzyme that has demonstrated usefulness as an IHC marker of hepatocellular differentiation. Previous studies have reported the efficacy of HepPar-1 (HP-1) and glypican-3 (GPC-3) IHC in liver FNA cytology. To our knowledge, no studies using AG-1 IHC have been performed on FNA specimens, and its performance characteristics have not yet been compared with HP-1 and GPC-3 IHC.

Design: IHC for AG-1, HP-1, and GPC-3 were performed on cell block sections from 92 liver FNAs including HCC (n=44), cirrhosis (n=2), focal nodular hyperplasia (FNH) (n=3), hepatic adenoma (HA) (n=2), dysplastic nodule (DN) (n=6) and metastatic carcinoma (MC) to the liver (n=35). Sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) for the cytology results were calculated for all immunomarkers in detecting HCC from non-HCC lesions.

Results: AG-1 IHC staining was generally intense and expressed more frequently in HCC (84.1%) compared with HP-1 (72.7%) and GPC-3 (56.8%). AG-1 and HP-1 expression was also observed in all cases (100%) of cirrhosis, FNH, HA, and DN, while GPC-3 expression was absent. All three immunomarkers were negative in MC.

AG-1, HP-1, and GPC-3 expression in HCC and non-HCC lesions

	HCC (n=44)	Other (n=48)
AG-1	37 (84.1%)	14 (29.2%)
HP-1	32 (72.7%)	14 (29.2%)
GPC-3	25 (56.8%)	0 (0%)

Sensitivity, Specificity, PPV, and NPV of AG-1, HP-1, and GPC-3 in distinguishing HCC from non-HCC lesions

	AG-1 (%)	HP-1 (%)	GPC-3 (%)
Sensitivity	84.1	72.7	56.8
Specificity	70.8	70.8	100
PPV	72.5	69.6	100
NPV	82.9	73.9	71.6

Conclusions: The results of the study demonstrate that both AG-1 and HP-1 are effective IHC markers of hepatocellular differentiation. AG-1 demonstrates superior sensitivity compared with HP-1 and GPC-3 in the diagnosis of HCC. GPC-3 demonstrates superior specificity. These results suggest that the utilization of AG-1 and HP-1, in combination with GPC-3, can aid in the diagnosis of HCC and be used to distinguish HCC from MC.

401 Implementation of BD FocalPoint GS in Clinical Practice: Impact on Human Papillomavirus (HPV) Rates and Biopsy Diagnoses

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Background: The the location-guided imaging system, FocalPoint GS (FPGS) has been recently implemented in clinical use on SurePath Pap tests in a number of institutions, including ours. However, in contrast to the Thinprep Imaging System (TIS), the impact of which has been studied extensively, the clinical performance of FPGS has been, to our knowledge only been the subject of a single study, which found an increase in ASC-US rates after its implementation. The aim of this study was to evaluate the impact of FPGS on the frequency of HPV and follow-up biops rates in ASC-US and negative Pap tests in women over 30 (NILM>30) before and after implementation of FPGS.

Design: We identified all cases diagnosed from 1/1/2007 to 3/30/2011 as ASC-US or NILM>30 that had reflex HPV testing performed by a PCR-based method using MY09/11 consensus primers and typing by RFLP. All cases were diagnosed by 2001 Bethesda System criteria on Surepath Pap tests. FPGS was implemented in our laboratory on 10/1/2010; all cases tested after this date constuituted the POST-FPGS chort, while the cases diagnosed before this date consituted the PRE-FPGS cohort.
The prevalence of any HPV type, high-risk HPV types (HR-HPV) and HPV16/18 and the frequency of abnormal follow-up biopsies diagnosed as CIN1 and above (CIN1+) and CIN2 and above (CIN2+) in the tow cohorts was compared statistically.

Results: During the study period, the ASC-US rate increased from 5.5% (12,464/227972) to 7.4%(4,234/57254. 13999 women with ASC-US and 15403 women with NILM>30 had HPV tests performed in our institution.

	AGE (mean±SD)	Any HPV (n,%)	HR-HPV (n,%)	HPV 16/18 (n,%)	BX rate	CIN1+ (% of BX)	CIN2+ (% of BX)
PRE-FPGS ASC-US (n=11551)	36.49±13.26	4690 (40.6%)	2054 (17.8%)	1006 (8.7%)	1764 (15.3%)	790 (44.8%)	278 (15.8%)
POST-FPGS ASC-US (n=2448)	38.77±12.91	918 (37.5%)	395 (16.1%)	177 (7.2%)	389 (15.9%)	181 (46.5%)	48 (12.3%)
PRE vs. POST p value	<0.0001	0.005	NS (0.053)	0.016	NS	NS	NS (0.1)
PRE-FPGS NILM>30 (n=12981)	45.3±10.7	924 (7.1%)	271 (2.1%)	134 (1%)	122 (0.9%)	14 (11.5%)	5 (4.1%)
POST-FPGS NILM>30 (n=2422)	45.5±10.5	181 (7.5%)	50 (2.1%)	24 (1%)	17 (0.7%)	5 (29.4%)	0 (0%)
PRE vs. POST p value	NS	NS	NS	NS	NS	NS (0.059)	NS

Conclusions: We found that the increase in the ASC-US rate was due to the detection of more cases in older women, which resulted in a decrease in the overall HPV positivity rate. However, the similar CIN1+ and CIN2+ rates are reassuring, suggesting that the increase in ASVC-US diagnoses is not due primarily to overcall. We found no changes in HPV rates or follow-up biopsy diagnoses in women with NILM>30.

402 Cytology and Pitfalls of EUS Sampling of Ectopic Splenic Tissue

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Background: Ectopic splenic tissue is common and can occur in a variety of sites but most frequently the splenic hilum. Although often noted as incidental masses on imaging studies including endoscopic ultrasound (EUS), ectopic splenic tissue may not be recognized if outside the usual splenic hilum due to variable EUS characteristics. EUS-guided fine needle aspiration (EUS-FNA) is used to evaluate abdominothoracic masses and ectopic spleen may occasionally be sampled. Cytologic recognition of ectopic spleen at the time of EUS may be difficult due to the relative rarity of splenic aspirations in most practice settings as well as the varied clinical features. Knowledge of the clinical, EUS, and cytologic features of ectopic splenic tissue is important for the cytologist interpreting EUS-FNA's.

Design: A retrospective search of the laboratory information system (LIS) identified 35 aspirations of spleen over 10 years including 10 EUS FNA cases with ectopic splenic tissue. The 10 cases included 6 males and 4 females with a mean age of 47.7 years. Two cases had corresponding surgical pathology material.

Results: Ectopic splenic tissue sampled by EUS FNA was located within the pancreas (4), peripancreatic (3), intrahepatic (2), and within the gastric wall (1). Size ranged from 0.5 to 3.5 cm (mean 1.77 cm) with a variety of EUS echo intensities ranging from hypoechoic to hyperechoic. Intraprocedural preliminary cytologic diagnoses were varied with two cases suspicious for neoplasm. The majority (8) were noted to be lymphoid tissue with no cases interpreted as splenic tissue onsite. The smears were variably cellular with a mix of small lymphocytes and tissue fragments. Tissue fragments commonly showed crush artifact and were composed of a mix of spindle cells and lymphocytes. Immunohistochemistry was performed in two cases with coexpression of CD8 and vascular markers CD31 or CD34.

Conclusions: 1. EUS FNA sampling of ectopic spleen is often located away from the splenic hilum and may be do to less frequent recognition by EUS, atypical EUS features, and/or overlapping imaging characteristics with other lesions such as lymph nodes and pancreatic neuroendocrine tumors.

2. Two cases were given preliminary diagnoses of suspicious for neoplasm which highlights the potential pitfall in the cytologic interpretation of ectopic splenic tissue.

3. Accurate diagnosis is facilitated by awareness of the varied locations of ectopic splenic tissue, knowledge of the cytologic appearance of the spleen, and communication with the endoscopist.

4. In some cases, immunohistochemistry may be a useful adjunct in confirming the cytologic diagnosis.

403 ProEx C as an Adjunct Molecular Marker To Improve the Detection of Urothelial Carcinoma

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Background: ProEx C is a novel antibody targeting the expression of topoisomerase II-alpha and minichromosome maintenance protein-2. Both proteins are overexpressed in the cell nucleus during aberrant S-phase induction of the neoplastic and HPV infected cells. Other studies have demonstrated ProEx C as an adjunct marker for assessing dysplasia in gynecologic specimens especially in the cervical biopsies and smears. A significant number of urine cytology specimens are diagnosed as "atypical" that triggers additional procedures that may result in patient discomfort and/or increase in cost. This study was designed to determine the utility of ProEx C in urine cytology samples for improving the detection of urothelial carcinoma.

Design: Sixty urine cytology specimens (12 negative, 35 atypical, and 13 positive cases) from July 2010-June 2011 which had a surgical follow up were retrieved. Residual fluid from the liquid-based ThinPrep urine specimen was used to make an additional slide for immunohistochemical stain. Smears were screened to confirm adequacy and cytologic diagnosis. ProEx C was recorded as positive in cytology samples when nuclear staining was seen in morphologically atypical urothelial cells and as negative if no staining was observed.

Results: A positive ProEx C stain had a sensitivity of 78.4%, specificity of 87%, PPV of 90.6% and NPV of 71.48% for the detection of urothelial carcinoma. Using McNemar-Fisher exact Chi-square analysis in a 2x2 table, a *P*-value of 0.016 was obtained when the ProEx C positive and negative stains compared in positive and negative follow ups in the cases diagnosed as "atypical" in Tables 1 & 2.

Table 1. Results of ProEx C stain in urine cytology specimen

Stain	Cytology Diagnoses (n=60)		
	Negative (n=12)	Atypical (n=35)	Positive (n=13)
ProEx C +	0	20	12
ProEx C -	12	12	1

Table 2. Results of ProEx C staining with follow-up diagnoses (n=60)

Stain	Negative cytology (n=12)		Atypical cytology (n=35) Chi-Sq P-value = 0.016		Positive cytology (n=13)	
	Negative follow-up	Positive follow-up	Negative follow-up	Positive follow-up	Negative follow-up	Positive follow-up
ProEx C+	0	0	3	17	0	12
ProEx C-	12	0	8	7	0	1

Conclusions: Based on this study, as outlined in the tables, ProEx C stain can be a useful adjunct test to urine cytology even with limited specimens. Significant difference was seen for positive Proex-C stain in atypical cases having a positive follow up versus negative follow up. Therefore, this test may assist in further classification of "indeterminate" or "atypical" diagnoses in urine smears.

404 Molecular Genetic Findings in Pediatric Thyroid Fine Needle Aspirations: Experience from a Large Academic Medical Center

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Background: The Bethesda System for Reporting Thyroid Cytopathology (TBSRTC) and its management guidelines for thyroid fine-needle aspiration (FNA) are largely based on data from studies with adult populations. Although thyroid nodules are more commonly identified in adults, the rate of malignancy in thyroid nodules in children is higher. Thus, our aim was to analyze the mutational profiles of pediatric thyroid cancer detected by molecular analysis of thyroid FNAs, and to examine its diagnostic use in thyroid nodules from children.

Design: FNAs from children (ages ≤ 21 years) were identified from our pathology archive during a 4.5 year period (2007-2011). Cases were categorized based on the diagnostic categories from TBSRTC and the findings were correlated with histological follow-up and molecular analysis performed for the following mutations: BRAF, NRAS61, HRAS61, KRAS12/13, RET/PTC and PAX8/PPARγ.

Results: A total of 179 cases from 142 patients were identified, including 96 cases (54%) with histological follow-up and 66 cases (37%) with molecular data. Of the 66 FNAs with molecular data, there were 47 (71%) negative, 8 (12%) indeterminate, and 11 (17%) positive for mutations. The FNA samples with molecular testing positive for any mutation were all papillary thyroid carcinomas (PTCs) on resection. The molecular findings in these FNA cases included 4 RAS mutations (36.5%; 1 HRAS, 2 NRAS, and 1 with HRAS and NRAS), 3 RET/PTC rearrangements (27.5%), 2 BRAF mutations (18%), and 2 PAX8/PPARg rearrangements (18%). The FNA diagnoses in these positive cases included atypia of undetermined significance (2 cases), suspicious for follicular or oncocytic neoplasm (5 cases), suspicious for PTC (1 case), and positive for PTC (3 cases).

Conclusions: To our knowledge, this is the first report of molecular testing for a panel of mutations performed in FNA samples from pediatric thyroid nodules. These data reveal 17% positivity for mutations in this population, which is higher than that seen in adult populations. The presence of any mutation in this study correlated with malignancy in 100% of cases, including nodules with indeterminate cytology. Our findings also demonstrate a higher prevalence of RAS and RET/PTC mutations and lower prevalence of BRAF mutations as compared to adult populations, which correlates with the less aggressive nature of PTCs in young individuals.

405 Grading and Staging of Pancreatic Endocrine Tumor: EUS-FNA-Based Compared to Surgical Pathology

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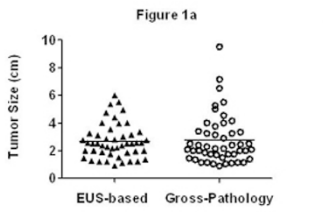
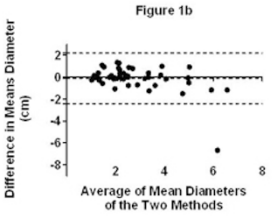
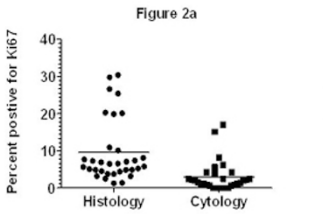
Background: A TNM system for staging and grading of Pancreatic endocrine neoplasms (PENs) has been proposed and shown to stratify the risk of PENs. The aim of this study is to assess if EUS-FNA-based TNM satging and staging correlates with that of surgical pathology.

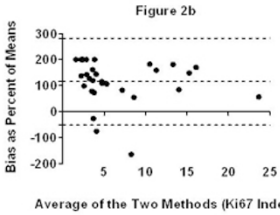
Design: This a retrospective study in which we reviewed medical records, cytology, histology EUS reporst trying to identify cases with adaquate follow-up. Maximum and minimum tumor diameters were extracted from EUS and the surgical pathology reports. Grading was based on Ki67 immunohistochemistry on the cell blocks and surgical specimens. The most proliferative area was photographed and scored using image Immunoratio™ program. Paired t-test was used to compare means of tumor diameters and Ki67 index. Bland-Altman (B-A) plot and kappa statistics were used to assess the differences and agreements between EUS-FNA-based vs. surgical pathology-based staging and grading of PENs.

Results: Of 138 patients with PENs, 61(44%) had surgical resection of whom 48 (79%) had their tumor measurement recorded and 30 (49%) had adequate material for Ki67 staining. The mean (SE) tumor diameter based on surgical pathology, 2.8 (0.26) cm, was not significantly different than that based on EUS, 2.7 (0.18) cm, Figure 1a. The B-A difference between the two methods was 0.1 (95% C.I. -2.4, 2.2), Figure 1b and there was a moderate agreement between the methods regarding staging, kappa statisitics 0.44. The mean (SE) Ki67 index based on histology was 9.6% (1.5%) significantly different than that based on cytology 3.0% (0.7%), p value .01, Figure 2a. The B–A difference between the two methods was 7.0 (95% C.I., -9.4, 23) and it varies with an increasing Ki67 index. This trend was attenuated when the difference was represented as a percentage of the means of the methods, Figure 2b. The two methods have poor agreement on PENs grading, kappa statistic 0.05.









Conclusions: There is moderate agreement between EUS-FNA-based and surgical pathology staging of PENs but poor agreement between the two methods on grading. There is a need for standardization of Ki67 staining and counting for both histology and cytology.

406 Evaluation of Atypical Urine Cytology Progression to Malignancy: An Eleven-Year Retrospective Review

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Background: In urine cytology, the diagnosis of atypia is subjective and clinical management based on these results can be difficult to determine. In this study, we determined the percentage of atypical urine diagnoses that progressed to positive cytology or surgical pathology results over an eleven year period.

Design: In a retrospective review of urinary tract specimens at our institution, we identified 1320 atypical urine cytology diagnoses from 851 patients performed from January 2000 through December 2010. We then reviewed all subsequent pathology reports to determine which patients developed positive cytology/surgical pathology diagnoses. In total, 4106 cytology and surgical pathology specimen reports were reviewed.

Results: At our institution, 8.1% (1320 of 16299) of urine cytology specimens were diagnosed as atypical during the eleven year period. Overall, 271 of 1320 initial atypical urine specimens (21%) progressed to positive cytology or surgical pathology results, with a mean time to progression of 155 days and median time of 43 days. The mean patient age at first atypical cytology diagnosis was 66 years (range 15 to 99 years) consisting of 604 male and 247 female patients.

Of the cases that progressed to malignancy, 118 were high grade (HG) (including high grade urothelial carcinoma, carcinoma in situ, invasive carcinoma, and urothelial metastasis) and 92 were low grade urothelial carcinoma (LG). Furthermore, 17 were found to be other primary cancers/metastasis (9 prostate, 7 renal clear cell, and 1 lung) and 44 cases had positive cytology results with no positive surgical pathology follow-up.

Rate of Atypia in Urinary Tract Specimens and Progression to Malignancy

	# Atypical (n)	% Atypical	# Progressed to Positive (n, %)	# Progressed to LG Urothelial Carcinoma (n)	% LG	# Progressed to HG Urothelial Carcinoma (n)	% HG
Bladder Barbotage/ Washing	869	8.9%	189 / 22%	74	39%	81	43%
Voided Urine	291	8.7%	41 / 14%	12	29%	17	41%
Upper Tract Urine	82	3.8%	31 / 38%	5	16%	17	55%
Urinary Diversion	60	16%	7 / 12%	0	0%	2	29%
Catheterized Urine	18	5.2%	3 / 17%	1	33%	1	33%
Total	1320	8.1%	271 / 21%	92	34%	118	44%

Conclusions: The rate of atypia in urine specimens at our institution is 8.1%. Of the specimen types, atypia was most commonly seen in urinary diversion specimens (16%) and the least common in upper tract cytology (3.8%). When diagnosed as atypical, upper tract specimens had the highest percentage of progression to high grade carcinoma. Therefore we postulate that the diagnosis of atypia in this specimen group has higher clinical significance and should be more aggressively managed.

407 Cell Block Cellularity Correlation with Clinico-Pathologic Variables in Pancreatic Neoplasms

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Background: Diagnosis of pancreatic tumors relies heavily on EUS guided FNA diagnosis. Thus far, procuring sufficient material for diagnosis has been prioritized, and thus, adequacy assessment mainly addresses material for diagnosis, with collection of cell block material mainly for potential immunohistochemistry for diagnosis purposes. With the advent of effective neo-adjuvant therapy for some cancers, and the growing field of personalized medicine, emphasis needs to shift to obtaining adequate material in the pre-operative setting. This study aims to correlate cellularity of cell block material with several clinicopathologic variables in an attempt to ascertain optimal conditions for adequate material for pre-treatment or theranostic testing.

Design: Cytology cases of pancreatic neoplasms diagnosed at 2 hospitals (H1,H2) within our institution, that had cell blocks prepared were identified over a 6 month period. Only cases with a diagnosis of neoplasm/ malignancy were included, excluding all cases with an indeterminate diagnosis. Most EUS-FNAs used 25 gauge needles, some used 22 or 19 gauge needles. Cell block cellularity (CBC) was re-evaluated by a single cytopathologist and graded (scale of 1 to 4). Clinicopathologic variables included location, EUS-FNA operator, number of passes, EUS size of mass and on-site evaluation.

Results: There were a total of 90 cases -72 adenocarcinoma, 15 neuroendocrine tumors and 9 others at H1 and H2.

Overall CBC was significantly higher at H1 ($p = 0.0123$).

CBC was significantly associated with onsite adequacy evaluation ($p = 0.037$).

CBC varied significantly between operators.

Mass size did not significantly affect CBC, when size cutoffs of 1cm or 3cm were used. Neither the total number of passes nor the needle gauge affected CBC.

Conclusions: When comparing CBC for pancreatic tumors against clinico-pathologic variables, mass size, total number of passes and needle gauge did not show significant differences. However, CBC varied significantly with presence of on-site evaluation, between two hospitals and between FNA operators demonstrating that a combination of technical variables (pathologist involvement/onsite evaluation) and operator skill/ experience and possibly other practice patterns between hospitals impacts CBC and in turn, adequacy for important ancillary/ theranostic tests.

408 Atypical Squamous Cells of Undetermined Significance (ASC-US) Associated with Atrophy in Liquid-Based (Surepath) Pap Tests: Prevalence of Human Papillomavirus Infections and Follow-Up Biopsy Diagnoses

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Background: Despite its use for more than two decades and the clearly formulated Bethesda system diagnostic criteria, the diagnosis of atypical squamous cells of undetermined significance is still the most challenging and least reproducible diagnosis made on Pap tests. The diagnostic challenges are greatly exaggerated by the presence of atrophic changes (ATR) in the Pap test. The aim of this study was to determine the human papillomavirus (HPV) and follow-up biopsy correlates on ASC-US+ATR.

Design: We identified all cases diagnosed from 1/1/2003 to 12/31/2010 as ASC-US with concomitant atrophic changes (ASC-US+ATR) that had reflex HPV testing performed by a PCR-based method using MY09/11 consensus primers and typing by RFLP. All cases were diagnosed by 2001 Bethesda System criteria on Surepath Pap tests. The prevalence of any HPV type, high-risk HPV types (HR-HPV) and HPV16/18 and the frequency of abnormal follow-up biopsies diagnosed as CIN1 and above (CIN1+) and CIN2 and above (CIN2+) was compared statistically to those of all other cases of ASC-diagnosed within the same interval.

Results: Of the 23077 cases diagnosed as ASC-US during the study interval, 284 (1.23%) were associated with ATR.

	AGE (MEAN+/- SD)	ALL HPV TYPES	HR-HPV TYPES	HPV TYPES 16/18	BIOPSY RATE	CIN1+	CIN2+
ALL ASC-US (N=23077)	36.3+/-13.2	8937 (38.7%)	4277 (18.5%)	1993 (8.6%)	4241 (18.4%)	1845 (8%)	581 (2.52%)
ASC-US+ATR (N=284)	59.4+/-10.4	39 (13.7%)	17 (6%)	3 (1.1%)	22 (7.8%)	5 (1.8%)	2 (0.7%)
ASC-US+ATR VS. ALL ASC-US %CHANGE		-125%	-67.7%	-87.8%	-57.9%	-106.2%	-101.8%
ASC-US+ATR VS. ALL ASC-US p VALUE	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	NS (0.053)

Women with ASC-US+ATR were significantly older than women with ASC-US without atrophy. However, the differences in prevalence of HPV, HR-HPV, HPV16/18 and of CIN1+ and CIN2+ diagnoses persisted even when the results were analyzed by age groups.

Conclusions: In our institution, ASC-US+ATR constitues a small fraction of ASC-US diagnoses.

We found profoundly and significantly lower HPV rates and follow-up abnormal biopsy diagnoses in women with ASC-US+ATR as compared to all women with a diagnosis of ASC-US.

Our data suggest that better criteria are needed to differentiate between atrophic changes and clinically significant squamous atypia in Pap tests.

409 Atypical Squamous Cells of Undetermined Significance (ASC-US) Associated with Atypical Repair in Liquid-Based (Surepath) Pap Tests: Prevalence of Human Papillomavirus Infections and Follow-Up Biopsy Diagnoses

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Background: Despite its use for more than two decades and the clearly formulated Bethesda system diagnostic criteria, the diagnosis of atypical squamous cells of undetermined significance (ASC-US) is still the most challenging and least reproducible diagnosis made on Pap tests. ASC-US associated with atypical reparative changes (ASC-US+ATYREP) is one of the subtypes of ASC-US recognized by the Bethesda System. Studies that have addressed the question of the significance of ASC-US+ATYREP have arrived to conflicting results, some authors even arguing against retaining this diagnostic category under ASC-US. The aim of this study was to determine the human papillomavirus (HPV) and follow-up biopsy correlates on ASC-US+ATR in an attempt to shed further light on this issue.

Design: We identified all cases diagnosed from 1/1/2003 to 12/31/2010 as ASC-US with atypical repair (ASC-US+ATYREP) that had reflex HPV testing performed by a PCR-based method using MY09/11 consensus primers and typing by RFLP. All cases were diagnosed by 2001 Bethesda System criteria on Surepath Pap tests. The prevalence

of any HPV type, high-risk HPV types (HR-HPV) and HPV16/18 and the frequency of abnormal follow-up biopsies diagnosed as CIN1 and above (CIN1+) and CIN2 and above (CIN2+) in women with ASC-ATYREP was compared statistically to those of all other cases of ASC-US diagnosed within the same time interval.

Results: Of the 23077 cases diagnosed as ASC-US during the study interval, 188 (0.81%) were associated with ATYREP.

	AGE (MEAN ±SD)	ALL HPV TYPES	HR-HPV TYPES	HPV TYPES 16/18	BIOPSY RATE	CIN1+	CIN2+
ALL ASC-US (N=23077)	36.3±13.2	8937 (38.7%)	4277 (18.5%)	1993 (8.6%)	4241 (18.4%)	1845 (8%)	581 (2.52%)
ASC-US+ATYREP (N=188)	38.9±13.6	46 (24.5%)	23 (12.2%)	19 (10.1%)	29 (15.4%)	10 (5.3%)	3 (1.6%)
ASC-US+ATYREP VS. ALL ASC-US %CHANGE		-36.8%	-34%				
ASC-US+ATYREP VS. ALL ASC-US p VALUE	0.007	<0.0001	0.029	NS	NS	NS	NS

Women with ASC-US+ATYREP were slightly older than other women with ASC-US, which most likely contributed to their lower HPV rates.

Conclusions: In our institution, ASC-US+ATYREP is a rare diagnosis and contributes to only a small fraction of ASC-US diagnoses.

We found lower overall HPV rates but similar HPV16/18 and abnormal follow-up biopsy rates in women with ASC-US-ATYREP compared to all ASC-US cases. Our results suggest retaining this diagnostic category under ASC-US.

410 Fine Needle Aspiration of Spleen Lesions: Cytopathologic Analysis of 66 Cases with Clinical and Histological Correlation

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Background: While commonly used in developing countries for evaluation of splenic involvement by infectious or systemic diseases, fine needle aspiration (FNA) of the spleen is rarely performed in North America. Recent increasing awareness of its utility in evaluating splenic lesions is accompanied by improved imaging detection and image-guided sampling techniques. Diagnostic issues arise due to the rarity and variety of the entities in the spleen.

Design: A retrospective review (2004-2011) of 66 splenic FNA samples from two academic centers was performed. FNAs were done under CT, ultrasound, endoscopic ultrasound, or MRI guidance or by direct aspiration during surgery. Clinical data, radiologic features, and follow up histologic/clinical findings were reviewed and correlated.

Results: Sixty-six splenic FNAs were performed on 30 female and 35 male patients ranging in age from 21 to 80 years (mean 51 years). One patient was biopsied twice for evaluation of a large splenic cyst. Thirty-eight (58%) patients had a prior history of malignancy including lymphoma/leukemia (15), carcinoma (16), neuroendocrine tumor (2), melanoma (2), germ cell tumor (2), and sarcoma (2). One patient had a history of both carcinoma and lymphoma. Ten cases were diagnosed as malignant. Of these, 6 were lymphomas, 3 metastatic carcinomas, and 1 histiocytic sarcoma. All 5 suspicious diagnoses were concerning for non-Hodgkin lymphoma. Forty-two (64%) cases were diagnosed as benign including granulomatous inflammation (9), vascular lesion (4), splenic epithelial cyst (3), extramedullary hematopoiesis (2), infection (2), and splenic tissue without specification (22). Five cases were diagnosed as atypical and 4 cases were reported as non-diagnostic. Thirty-seven cases had associated surgical core biopsy and/or resection specimens. There were 3 false negative cases due to sampling error and 1 false positive case due to over interpretation of cellular benign splenic tissue as a mesenchymal neoplasm. There were no complications associated with the FNA procedure.

Conclusions: FNA of splenic lesions is a safe and useful tool to document recurrent lymphoma or metastatic carcinoma. Although more than half of the patients presented with a prior history of malignancy, the majority of the lesions turned out to be benign. Therefore, it is important to be familiar with the spectrum of cytologic findings of this uncommonly aspirated organ to avoid unnecessary splenectomies.

411 EUS-FNA in the Diagnosis of Pancreatic and Peri-Pancreatic Lymphoma

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Background: Pancreatic and peri-pancreatic lymphomas are rare tumors that may present clinically as pancreatic adenocarcinomas do. Endoscopic ultrasound-guided fine-needle aspiration (EUS-FNA) has recently played an increasing role in the diagnosis of non-Hodgkin lymphoma, especially when combined with ancillary studies including flow cytometry and immunohistochemistry (IHC). However, the utility of EUS-FNA in the diagnosis of pancreatic lymphoma has rarely been reported.

Design: A retrospective search was conducted at our institution on all pancreatic and peri-pancreatic EUS-FNA specimens from January, 2000 - December, 2010. Out of 2,397 pancreatic EUS-FNA specimens identified, 27 were aspirates of a lymphoproliferative process including lymphoma and atypical lymphoid population. Pap stains, Diff-Quik stains, cell block, and IHC stains, where available, were evaluated. All available histology specimens and flow cytometry data were also evaluated.

Results: During the study period, 27 aspirates from 24 patients received diagnoses of lymphoma (25) or an atypical lymphoid population (2). Twenty-five lymphoma aspirates from 22 patients, including 11 men and 11 women, aged 33-85 (mean age of 63.2 years), were evaluated. Aspirates were derived from the pancreas in 12 patients (55%) and from a peri-pancreatic lymph node or mass in 10 patients (45%). Fifteen patients (68%) presented with a primary pancreatic lymphoma while 7 patients (32%) had secondary extension from a non-pancreatic primary.

Pathologic Features of Pancreatic and Peri-Pancreatic Lymphomas

Characteristic	**N Patients (%)**
EUS-FNA Diagnosis	
Negative	1 (4.5%)
Atypical	12 (54.5%)
Suspicious	3 (13.6%)
Positive	6 (27.3%)
Flow Cytometry Available for EUS-FNA Specimen	16 (72.7%)
Histologic Confirmation of Lymphoma	10 (45.5%)
Final Diagnosis*	
Chronic lymphocytic leukemia/small lymphocytic lymphoma	3 (13.6%)
Diffuse large B-cell lymphoma	7 (31.8%)
Lymphoma, follicular center cell origin	5 (22.8%)
Marginal zone lymphoma	3 (13.6%)
T-cell/histiocyte rich large B-cell lymphoma	1 (4.5%)
B-cell lymphoma, not otherwise specified	3 (13.6%)

* Final diagnosis based on all data available per case including cytologic, flow cytometric and histologic diagnoses.

Conclusions: EUS-FNA with flow cytometry is a reliable tool in diagnosing pancreatic and peri-pancreatic lymphoma, underscoring the value of having a cytopathologist in the endoscopy suite for a rapid interpretation of these lesions. When lymphoma is suspected, additional passes dedicated to flow cytometry are warranted.

412 Cytopathology of Exra-Cranial Meningiomas: Study Involving 11 Ectopic and Metastatic Meningiomas

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Background: Although rare outside of the cranium, meningiomas can be seen throughout the body either as ectopic or metastatic tumors. The cytomorphology of these neoplasms is not well documented. The rarity of such lesions and the fact they may exhibit morphological characteristics that overlap with other tumors poses a significant diagnostic challenge. The aim of this study is to characterize the cytomorphology of ectopic and metastatic meningiomas with histologic correlation in 11 cases.

Design: A retrospective analysis of 8 primary ectopic and 3 metastatic meningiomas with cytologic preparations [5 fine needle aspirations (FNA) and 4 intraoperative smears/touch preparations] were collected from our laboratory information system or collaborators (2 FNA cases) and correlated with available surgical resection specimens. Data regarding clinical findings, cytomorphology, histologic features and immunostaining (IHC) were recorded and analyzed.

Results: Patients were on average 50 years of age (range 30-71) with a male:female ratio of 1:3. Two of the 3 patients with metastases had a previous diagnosis (2/4; 50%). Metastases were diagnosed in the lung (2/3; 67%) and liver (1/3; 33%). Primary ectopic meningiomas were located in the sinuses and ear (4/8; 50%), orbit (2/8; 25%), and neck (2/8; 25%). The 4 cases associated with on-site evaluation were deferred (2 favored meningioma, 1 epithelioid neoplasm, and 1 atypical). Cytomorphologic features characteristic of meningiomas included clusters of spindled cells arranged in whorls (4 cases), intranuclear inclusions (5 cases), nuclear grooves (5 cases) and psammomatous calcification (3 cases). Unusual cytomorphologic features included epithelioid cell predominance (2 cases), abundant inflammation (2 cases), small cell change (1 case), papillary (1 case) and pseudoacinar growth (1 case). Metastatic tumors all had nuclear atypia (3/3; 100%) and mitoses (1 case) or necrosis (1 case). In 6 cases where IHC was performed, positive stains included EMA (100%), vimentin (100%) and pankeratin (33%); and all tumors were S-100 negative. High-grade features were seen in surgical resections of all metastases (one grade 2, two grade 3) and one ectopic meningioma (grade 2).

Conclusions: Metastatic and primary ectopic meningiomas can be encountered in cytologic specimens. Such tumors should be included in the differential when characteristic cytomorphologic features of meningiomas are seen. Cytopathologists should be aware that these lesions could be mistaken for other tumors.

413 Frequency and Follow-Up Findings of Abnormal Cervical Cytology in Women ≥ 65 Years in a High-Risk Population

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Background: The 2009 ACOG guidelines for cervical screening recommend that the upper age limit for discontinuing screening be 65 or 70 years in those who have had ≥3 consecutive negative smears and no abnormal smear in the past 10 years; or have had total hysterectomy for non-cancer reason and no history of HSIL. This study reviews the abnormal cervical cytology findings in women ≥ 65 years to determine the disease burden in this age-group in a high-risk population.

Design: All abnormal cervical Pap smears (ASCUS, LSIL, ASC-H, HSIL, cancer) in women ≥ 65 years between January 2006 and June 2011 are extracted from the pathology data base and reviewed. Available follow up data of hrHPV profile, repeat cytology and histology are correlated with the abnormal findings.

Results: 145,144 Pap smears were received during the period with 5,700 (3.9%) from the target population (age range 65-89 years). 263 abnormal smears were identified from 209 women. Table 1 shows the distribution of abnormal cytology findings and corresponding biopsy/cytology follow-up. Of the 108 ASCUS index cases, 69 had HPV data with 26(37.7%) hrHPV+. Of the 13 ASCUS cases, carcinoma (3) and CIN2/3 (4) were diagnosed and invasive carcinoma in 14/48 cases (29.2%) with biopsy follow-up.

Distribution of Cytology Abnormality and Follow up Findings in Women More than 65 years

Index Cytology(No of Cases)	Follow up Cytology		Histology		hrHPV +VE	hrHPV -VE
ASCUS(108)	ASCUS	10	Atrophy	2	26(37.7%)	43(62.3%)
	LSIL	2	CIN1	4		
	ASC-H	1	CIN2/3	4		
	HSIL	4	Carcinoma	3		
	AGC	1				
	Carcinoma	0				
	Total	18	Total	13		
LSIL(49)	ASCUS	9	CIN1	6		
	LSIL	8	CIN2/3	2		
	HSIL	1	Carcinoma	1		
	Total	18	Total	9		
ASC-H(10)			CIN1	1	2	
HSIL(25)	LSIL	1	CIN1	2		
			CIN2/3	6		
			Carcinoma	4		
	Total	1	Total	12		
AGC(6)	ASCUS	1	CIN1	2		
			CIN2/3	1		
			Carcinoma	1		
	Total	1	Total	4		
Carcinoma(11)			CIN3	4		
			Carcinoma	5		
			Total	9		

Conclusions: The study shows significant carcinoma detection rate (29.2%) and hrHPV+ ASCUS cases (37.7%) in those with follow-up. Cervical screening is still desirably in women **≥65 y**ears in high-risk population.

414 Diagnostic Challenges of Pancreatic Cysts: A Proposal for a Multimodality Approach

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Background: The diagnostic distinction between mucinous and non-mucinous cystic lesions of the pancreas is clinically important as it has significant treatment implications. Mucinous cystic lesions include mucinous cystic neoplasm (MCN) and intraductal papillary mucinous neoplasm (IPMN), and require surgical excision. Non-mucinous lesions include serous cystic neoplasms and cystic neuroendocrine tumors and are managed with observation/follow-up. While EUS-FNA has emerged as the primary modality for obtaining an FNA sample from pancreatic lesions, cystic lesions present unique challenges, including undersampling and the treacherous gastrointestinal contamination. We investigated a combination of parameters: endoscopic impression, cytologic evaluation, mucin staining, and cyst fluid chemical analysis.The purpose of this study was to assess the utility of a multimodality approach in discriminating non-mucinous from mucinous lesions.

Design: All EUS-FNA pancreatic cysts cases performed at our institution from January 2009 to December 2010 were retrieved. Cases with mural nodules/solid masses were excluded and only cases with available surgical follow-up information were selected. The endoscopic findings, cytologic features, mucicarmine stains, and cyst fluid chemistry results were reviewed. The sensitivity and specificity of cytologic diagnosis were calculated using surgical follow-up as the gold standard.

Results: A total of 349 EUS-guided FNA procedures for pancreatic cystic lesions were performed. Of these, 65 fulfilled our criteria. Mean and median patient age were 62.0 and 67.0 years, respectively (range 31-84 years), 24 males and 41 females. Sensitivity, specificity, positive predictive and negative predictive values and accuracy were calculated for each parameter and combined parameters.

Parameters	N	Sensitivity	Specificity	PPV	NPV	Accuracy
FNA cytology	86	48.4%	100.0%	100.0%	51.5%	66.7%
Mucin stain	31	52.6%	88.9%	90.9%	47.1%	64.3%
CEA analysis	59	55.3%	81.0%	84.0%	50.0%	64.4%
EUS	70	83.3%	61.1%	81.1%	64.7%	75.9%
Combination test	26	94.7%	42.9%	81.8%	75.0%	80.8%

Conclusions: Our study highlights the diagnostic challenges of evaluating pancreatic cystic lesions and discriminating mucinous from non-mucinous lesions. We found that when used alone, endoscopic imaging was the most sensitive, while cytologic evaluation was the most specific. While no single test had acceptable performance characteristics, we demonstrate that using them in combination can significantly increase diagnostic accuracy.

415 Comparison of FNA and Core Biopsy Versus Complete Excision in Monitoring Tumor Response to EGFR Blockade in Murine Xenograft Models

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Background: Fine Needle Aspiration (FNA) is a routine diagnostic tool, however core biopsy (cbx) is more widely used in settings that require ancillary studies. There are very few reports comparing their ability to adequately sample heterogeneous lesional tissue. To test this, a system is necessary where the extent of heterogeneity can be known, then sampled using FNA or cbx, and referenced to a gold standard i.e. complete excision. The objective of this study was to determine whether FNA is an appropriate tool to measure selective response to erlotinib in murine heterogeneous tumor model.

Design: Two cell lines were used *in vivo*: A375 melanoma (resistant to erlotinib) and H292 lung carcinoma (sensitive). Three sets of tumors were made: pure A375, pure H292, and a mixture of H292 and A375. Tumor-bearing animals received erlotinib or vehicle. After the therapy, FNA and cbx were procured; the tumors excised *in toto* for complete cross-sections. All specimens were formalin-fixed and paraffin embedded, the slides stained for cytokeratin 7 and S100 protein by immunohistochemistry; a fraction positive was assigned (0-100%).

Results: The average scores for CK7 for pure melanoma were low for all three sampling techniques: excision 1%, core bx 6% and FNA 0%; the S100 scores were: excisions 87%, core bx 72%, and FNA 82%. For pure carcinoma CK7 scores were: excision 99%, core bx100% and FNA 98%; S100 scores were: excision 0%, core bx 2.5% and FNA 0%. Treatment had no effect. The scores of vehicle group, mixed tumors for S100 were: excision 7%, core bx 15%, FNA 6%. For the same tumors, CK7 scores were: excision 83%, core bx 91%, and FNA 94%. For the tarceva group the S100 scores were: excision 29%, core bx 19% and FNA 29%; CK7 scores were: excision 53%, core bx 66% and FNA 33%.

Conclusions: The scores of pure tumors showed that CK7 is more accurate than S100 in discriminating between carcinoma and melanoma. In the untreated mixed tumors, the scores for FNA samples were similar to the reference standard, while the cbx overestimated the S100-positve fraction. In the treated mixed tumors, FNA undercounted the proportion of CK7-positive cells compared to reference, but it was accurate in S100 determination. In contrast, the cbx overcounted the CK7-positve cells, and undercounted the S100 fraction. These data suggest that the sampling accuracy of FNA may be superior to that of core biopsy, and that FNA can find application in following tumor response in the context of human clinical trials.

416 Cost Analysis of Thyroid Fine Needle Aspiration (FNA) On-Site Evaluation (OSE)

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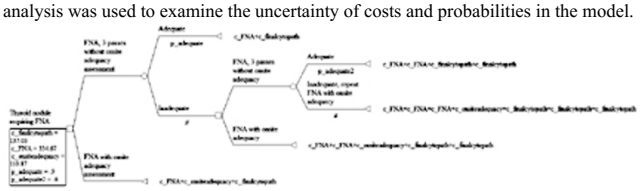
Background: Experienced operators with high adequacy rates for thyroid FNA may not benefit from OSE. OSE increases cost and length of procedure. Furthermore, in high volume centers, time, resource and reimbursement issues constrain the ability to provide OSE for every thyroid FNA. We hypothesized that rather than perform routine OSE, it would be less costly to limit the performance of OSE to repeat FNA after initial unsatisfactory FNA.

Design: A formal decision model was constructed using nested algorithms to compare strategies of routine OSE versus select OSE after unsatisfactory FNA. All FNAs with OSE were assumed to be adequate. Three passes were assumed to be performed for each FNA. Adequacy rates for FNA without OSE were estimated from contemporary institutional data and literature review. Medicare reimbursement data were used to estimate costs in 2011 USD in order to maintain a 3rd party payer perspective. Sensitivity analysis was used to examine the uncertainty of costs and probabilities in the model.



Results: OSE strategy had an overall cost of $582.57. FNA without OSE cost $529.96, producing cost savings of $52.61. Performance of initial FNA without OSE remained cost-saving until the adequacy rate of submitted samples fell to the threshold value of 81% or the cost of OSE fell to $52.41. Institutional data showed an unsatisfactory rate of 40% when an initial unsatisfactory FNA was re-attempted without OSE. The decision to perform OSE on all repeat FNAs produced cost savings of $122.16.

Conclusions: The strategy of routine OSE for all thyroid FNAs was more costly than selective OSE for initial unsatisfactory biopsies unless the initial adequacy rate was less than 81%. OSE after 1st unsatisfactory biopsy was the least costly selective OSE strategy as long as the repeat FNA inadequacy rate was less than 81%. This study was limited to the evaluation of cost only and does not take into account the factors of procedure length, delay in diagnosis, or patient time and discomfort.

417 Prospective Analysis of Atypical Epithelial Cells as a High Risk Cytological Feature for Malignancy in Pancreatic Cysts

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Background: By retrospective analysis, we have reported on the significance of atypical epithelial cells (AEC) in mucinous cyst fluids for detecting at least moderate dysplasia and predicting high-grade dysplasia (HGD) or worse, including in small branch-duct intraductal papillary mucinous neoplasms (IPMN). Here we report on the outcome of the prospective application of reporting AEC or worse (≥AEC) in EUS-FNA specimens of pancreatic cysts.

Design: All EUS-FNA of pancreatic cysts performed between Jan. 2006 and June 2011 were evaluated. Cytological, histological, imaging and cyst fluid CEA data were recorded. Performance characteristics of ≥AEC on cytology for predicting malignancy or mucinous cysts with high grade dysplasia was assessed. Nondiagnostic FNAs were excluded (no epithelial cells +/- CEA<192 ng/ml). Cysts were classified as mucinous on cytology with CEA >192 ng/ml or mucinous epithelium +/- extracellular mucin. Endocrine neoplasms are classified as malignant. Original reports were used. Atypical epithelial cells were defined as epithelial cells singly or in clusters with increased N/C ratio, nuclear hyperchromasia +/- membrane abnormalities and +/- cytoplasmic vacuoles, and not recognizable as GI contamination.

Results: A total of 404 EUS-FNAs were performed in 352 patients. The 70 patients with histological confirmation of diagnosis were analyzed using the FNA just prior to resection in patients with multiple FNAs of the same site; 4 aspirates were non-diagnostic and not included in the performance calculations (Table 1).

Histologically Confirmed Cysts (n=66)

	Malignant	Not Malignant
	(TP; n=20)	(FP; n=6)
≥AEC on cytology	PDAC (7)	IPMN-MD (4)
	IPMN-inv (5); HGD (4)	MCN-denuded (1)
	Cystic PanNET (4)	Non-neoplastic MC (1)
	(FN; n=4)	(TN; n=36)
	PDAC (2)	Pseudocyst (17)
<AEC on cytology	IPMN-HGD (1)	Serous cystadenoma (1)
	Cystic PanNET (1)	IPMN-LGD (5); MD (6)
		MCN-LGD (4); MD (3)

Sensitivity=83%; Specificity=86%; PPV= 77%; NPV= 90%; Accuracy =85%

If moderate dysplasia is considered a true positive with AEC cytology, specificity increases to 97%, but at the expense of sensitivity, 68%, and accuracy 80%.

Conclusions: Cytology is a specific and fairly accurate screening test for pancreatic cysts that require resection. AEC or worse on cytology predicts either a secondarily cystic solid neoplasm that requires resection, or a mucinous cyst with at least moderate dysplasia (specificity 97%), and suspicious for a mucinous cyst with HGD or worse (specificity 86%).

418 Utility of Cerebrospinal Fluid in the Diagnosis of Non-Hodgkin Lymphoma

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Background: Flow cytometric analysis (FCA) of cerebrospinal fluid (CSF) increases the likelihood of detecting central nervous system involvement by lymphoma when compared to cytopathologic examination alone. It has become routine practice, however, to submit CSF for FCA even when clinical suspicion for lymphoma is low. The purpose of this study is to assess the value of FCA with cytology as a screening tool in the work-up of patients with neurologic symptoms.

Design: Between June 2001 and June 2011, 368 patients had 496 CSF samples submitted for FCA and cytologic review. Electronic medical records were reviewed for patient symptomatology, history of malignancy, brain imaging, FCA results, cytology results, brain biopsy, and clinical follow-up, including autopsy results.

Results: Patients with neurologic symptoms but no history of hematologic malignancy made up 62% of patients. Thirty percent of patients had a history of lymphoma, and the remaining 8% were immunosuppresed. Most patients (93%) had brain imaging prior to lumbar puncture, and 52% of those had significant neurologic findings by imaging. Overall, FCA was positive in 22 cases (4%), negative in 280 (57%), and indeterminate in 194 (39%). Of the 22 cases positive by FCA, 12 (55%) were also positive by cytologic examination, but 9 (45%) were negative. Confirmatory brain biopsies were performed on 64 patients (17%). With open biopsy as the gold standard, the sensitivity and specificity of combined FCA and cytology are 24% and 96%, respectively. If one assumes that all positive FCA are true positives, as is done when making treatment decisions, the sensitivity/specificity of combined FCA and cytology increase to 64% and 96%, respectively. Patients with a positive FCA or brain biopsy were more likely to have either a history of hematologic malignancy or findings on brain imaging ($p<0.05$), making the positive predictive value of FCA in this population >95% despite the low sensitivity.

Conclusions: Although flow cytometric analysis of CSF is very specific for CNS lymphoma, the sensitivity of this test is too low for routine CSF screening of patients with neurologic symptoms. In an era of rising health care costs that necessitates judicious use of resources, FCA is not an appropriate initial test if the clinical suspicion for CNS lymphoma is low, and its use should be reserved for ancillary testing in the appropriate clinical setting.

419 How Useful Is Reflex HPV Testing in Patients with Atypical Glandular Cells of Undetermined Significance?

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Background: Cytologic interpretation of atypical glandular cells (AGC) indicates the presence of atypical glandular cells that mostly originate from the endocervix or the endometrium. Current guidelines recommend immediate colposcopy and endocervical with/or without endometrial sampling along with high risk human papiloma virus (HR-HPV) testing for follow up in patients with AGC. Recently, a few small studies suggested that HR-HPV DNA testing alone may also be used to triage patients with AGC. In the current study, we aim to investigate whether HR-HPV testing in the AGC population identifies patients with risk for cervical dysplasia/neoplasia.

Design: We retrospectively identified all patients with cervical cytology diagnosis of AGC within 61 consecutive months between Jan 2006 and Feb 2011. HR-HPV DNA test results and follow up biopsy data were analyzed.

Results: Of the 17,823 pap tests, 119 were reported as AGC and 1693 as atypical squamous cells (ASC), which corresponds to AGC to ASC ratio of 0.07. Ninety patients (75%) received a follow up biopsy. Thirty two (35%) patients had reflex HR-HPV testing of which 5 cases were HR-HPV positive, 26 cases were HR-HPV negative and 1 case had an equivocal results. Out of 32 cases, there were 24 (26%) patients who underwent HR-HPV testing and follow up biopsy. Four out of 24 patients (16%) who had AGC on pap smear and were negative for HR-HPV testing had cervical dysplasia or invasive squamous cell carcinoma. Positive predictive value of HR- HPV testing in AGC for detection of cervical dysplasia is 100%. Negative predictive value of HR-HPV negative for the absence of squamous dysplasia is 81%. The sensitivity of HR-HPV test is 42% and specificity is 100% in AGC.

Correlation of surgical biopsy and HR-HPV test results in patients diagnosed with AGC.

Biopsy diagnosis	**HPV +**	**HPV -**
Invasive SCC		1
CIN II-III	1	2
CIN I	2	1
Endometrial adenocarcinoma		4
Benign findings		13
No follow up biopsy	2	5
Total	**5**	**26**

Conclusions: While the positive HR-HPV testing in AGC patient strongly suggests cervical dysplasia, negative test results do not rule out significant cervical pathology. A higher than expected false negative rate and lower than expected sensitivity were noted. Our findings support direct colposcopy and biopsy instead of triage of an AGC cases with HR-HPV DNA testing.

420 The Benefits of a Repeat FNA in Follicular Lesion of Undetermined Significance (FLUS) Cases

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Background: Fine needle aspiration (FNA) is an important screening tool in the work-up of thyroid nodules. The management recommended by The Bethesda System for Reporting Thyroid Cytopathology (TBS) for a FLUS diagnosis is repeat FNA. As studies are limited, we sought to determine the benefit of a repeat FNA and the rate of malignancy in repeat FLUS cases.

Design: Retrospective review of all thyroid FNAs performed at UCLA Medical Center from 2003-2007 was performed. Cases that had a previous history of thyroidectomy or diagnosis of thyroid cancer were eliminated. Cases were re-reviewed and classified based on TBS. All cases categorized into FLUS were examined. Prior or subsequent repeat FNAs and surgical resection follow-up results were recorded for each nodule. Malignancy rates were calculated based on 1) excision and FNA diagnosis and 2) excision alone, and reported as a range between both rates.

Results: Of 2,972 thyroid FNA cases, 104 (3%) were classified as FLUS. The overall malignancy rate for all FLUS cases was 19-43%. 29 of 104 cases had a prior and/or repeat FNA. Malignancy rates did not differ significantly between repeat FLUS and non-repeat FLUS cases (17-56% and 20-41%, respectively). Prior and subsequent repeat FNA diagnoses are shown in Table 1. A repeat FNA reclassified 10 of 15 (67%) FLUS cases into a diagnostic category with definitive management, including 8 (53%) subsequent benign diagnoses (Table 1). Because case numbers were low, all prior/repeat FNAs were treated equally and combined for malignancy rate analysis (Table 2). Three of 4 FLUS+FLUS cases were malignant on excision.

TABLE 1: FLUS cases with prior/repeat FNA

Diagnostic category	Prior FNA	Repeat FNA
Non-diagnostic	1	0
Benign	8	8
FLUS	9	5
(Suspicious for) follicular neoplasm	0	0
Suspicious for malignancy	0	1
Malignant	0	1

TABLE 2: Malignancy rate for FLUS cases with repeat FNA

Repeat FNA Diagnosis	Total # of cases	# Excised	# Malignant	Malignancy rate
FLUS+ Non-diagnostic	1	0	0	-
FLUS+ Benign	13	3	1*	8-33%
FLUS+ FLUS	13	4	3	23-75%
FLUS+suspicious for malignancy	1	1	0	-
FLUS + malignant	1	1	1	100%

* - Benign FNA with a subsequent repeat FNA diagnosis of FLUS

Conclusions: Because of the high malignancy rate in FLUS+FLUS thyroid FNAs, these patients should undergo surgical excision. A repeat FNA may be beneficial because a majority of these cases are placed into a TBS category with definitive management. Further studies, including a cost-benefit analysis comparing repeat FNA with surgical excision for single FLUS cases, may be warranted.

421 Pancreatic Fine-Needle Aspiration Cytology in Patients < 35-Years of Age: A Retrospective Review of 175 Cases Spanning a 16-Year Period

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Background: Pancreatic lesions in young patients are relatively rare and, to our knowledge, the clinical value of pancreatic fine needle aspiration (FNA) in patients < 35 years of age has not been previously established by any other large retrospective studies.

Design: A computerized search of our laboratory information system was performed for the 16-year period (1994-2010) and all pancreatic FNA cases performed on patients < 35 years of age were identified. All FNA and all available correlating surgical pathology reports were reviewed.

Results: There were a total of 175 cases of pancreatic FNA performed on 111 males and 65 females under the age of 35 (range: 8-34, mean: 27 years). The FNA diagnoses included 39 malignancies, 114 negative for malignancy, 8 atypia, and 14 cases that were nondiagnostic. Of the 39 malignant FNA cases, the diagnoses included 19 pancreatic neuroendocrine tumors (PNET), 11 solid pseudopapillary neoplasms (SPN), 5 adenocarcinomas, 2 metastatic adenocarcinomas, 1 gastrointestinal stromal tumor, and 1 melanoma. Histologic follow-up was available in 22 of the 39 malignant cases (56%), and malignancy was confirmed in all cases. However, 1 case of SPN had been misclassified as PNET by FNA. Of the 114 FNAs diagnosed as negative for malignancy, 24 had histologic follow-up which included 9 cases of chronic pancreatitis, 6 benign cysts, 3 mucinous cystic neoplasms, 3 serous cystadenomas, 1 PNET, 1 focal epithelial atypia, and 1 with no pathologic change. Follow-up histology was available for 3

of the 8 cases diagnosed as 'atypia' by FNA and included 1 benign cyst, 1 chronic pancreatitis, and 1 serous cystadenoma. Histologic follow-up was available in 2 of the 14 nondiagnostic FNAs, both of which showed PNET. The overall sensitivity was 85% and the specificity was 100%.

Conclusions: The majority of the pancreatic lesions in patient under 35 years were benign with malignant neoplasms accounting for less than one quarter of the cases. The most common neoplasms in this age group included PNET, followed by SPN with both tumors accounting for 75% of all the neoplasms encountered in this age group. Mucinous cystic neoplasm was the most common cause of a false-negative FNA diagnosis. FNA is a clinically useful diagnostic test in patients < 35 years of age with pancreatic lesions.

422 Relative Sensitivity of Fine Needle Aspiration by Tumor Type and Size

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Background: Fine-needle aspiration (FNA) of papillary thyroid carcinoma has overall high sensitivity. Previous studies have shown correlation between FNA sensitivity and size of papillary thyroid carcinoma, and lower sensitivity for follicular variant of papillary carcinoma. Data on other types of thyroid carcinomas are lacking.

Design: We reviewed the results of 997 resected thyroid carcinomas at two institutions (5/1/2005 - 8/2011; 1/1999 to 11/2009), and correlated the findings with clinical and cytologic information. FNA diagnoses were classified according to The Bethesda System for Reporting Thyroid Cytopathology, and FNA diagnoses of "Malignant", "Suspicious for Malignancy", and "Suspicious for Follicular/Oncocytic Neoplasm" were considered "positive". Overall sensitivity of FNA and sensitivities based on size groups (≤1.0 cm, 1-3 cm, ≥3.0 cm) were calculated using "positive" FNAs as the numerator for each malignancy type.

Results: There were 868 papillary carcinomas (including 371 follicular variants), 101 follicular carcinomas (including 31 oncocytic variants), 16 medullary carcinomas, and 12 poorly differentiated carcinomas. The mean size of classical papillary carcinoma (1.9 cm, range 0.3-6.5 cm) was significantly smaller than for follicular variant of papillary carcinoma (2.2 cm, range 0.3-9.5 cm), follicular carcinoma, oncocytic variant (3.0 cm, range 0.4-10.0 cm), follicular carcinoma (3.0 cm, range 0.6-9.5 cm), and poorly differentiated carcinoma (2.8 cm, range 1.2-6.4 cm) ($p < 0.003$ for each), but not for medullary carcinoma (1.7 cm, range 0.6-3.3 cm) ($p = 0.45$). FNA sensitivities based on size groups are shown in Table 1.

Table 1: FNA Sensitivity by Tumor Type and Size

	≤1.0 cm	1-3 cm	≥3.0 cm	Overall Sensitivity
Papillary Carcinoma	88%	91%	77%	88%
Follicular Variant of Papillary Carcinoma	74%	79%	77%	78%
Follicular Carcinoma	67%	70%	72%	71%
Follicular Carcinoma, Oncocytic Variant	50%	83%	100%	84%
Medullary Carcinoma	100%	89%	100%	94%
Poorly Differentiated Carcinoma	N/A	100%	100%	100%

Conclusions: We conclude FNA is more sensitive overall for classic papillary carcinomas and medullary carcinomas, because they have characteristic cytologic features, compared to other thyroid malignancies. FNAs are less sensitive for small (≤1 cm) follicular based lesions. For follicular carcinoma and oncocytic (Hurthle) variant of follicular carcinoma, FNA sensitivity improves with size beyond 3 cm.

423 Cytologic Evaluation of Primary Bone Lesions Sampled by Fine Needle Aspiration Biopsy: Diagnostic Utility

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Background: Open biopsy is the traditional method for sampling bone lesions; however, this procedure puts the patients at risk for fracture and infection. A less traumatic approach is the image-guided needle biopsy technique, using either fine needle aspiration (FNA) or core needle biopsy (CNB) depending on the consistency of the lesion. These techniques yield less material and due to their rarity in clinical practice, pathologists have less experience interpreting these samples. This study analizes the diagnostic outcomes of FNA/CNB performed on bone lesions, with a special emphasis on primary bone tumors, in order to build evidence that a meaningful diagnosis can be rendered from these samples in most cases.

Design: All bone FNA/CNB (FNAB) performed with CT-guidance between 2006-2011 were reviewed. Site of involvement, method of collection, preliminary on-site interpretation, cytologic diagnosis, and follow-up were recorded.

Results: 220 bone FNABs were performed at our center during this interval. Biopsy sites were: pelvic bones (44.5%), long bones (23.1%), spine (20.4%), and other (12%). A useful interpretation was rendered in all but 2.7% nondiagnostic FNABs. 41 FNABs were from primary bone lesions: 73.1% CNB only, 24.4% CNB and FNA, and 2.5% FNA only. Touch imprints and smears provided useful diagnostic clues.

Table 1. Cytologic Findings in 41 Primary Bone Lesions FNABs

Cytologic interpretation (case numbers)	Findings on touch imprints/smear slides
Cartilage-forming tumors (13)	Chondroid matrix
Osteoid-forming tumors (2)	Rare osteoclasts and osteoblasts; no matrix
Ewing sarcoma (4)	Diagnostic material
Langerhans cell histiocytosis (LGH) (3)	Mixed inflammation, Langerhans cells present
Giant cell lesions (5)	Giant cells single or in groups
Fibro-osseous lesions (3)	Rare spindle cells
Sarcomas (3)	Diagnostic material
Glomus tumor (1), nerve sheath tumor (1)	Blood
Descriptive (6)	Blood or bone marrow elements

In conjunction with cell block, specific diagnoses were rendered in 35 cases (85.4%). On follow-up, the remaining 6 cases were: fibrous dysplasia, LCH, reactive histiocytic process, high-grade sarcoma, osteonecrosis; one case had no further evaluation. In 16 cases with surgical follow-up, histologic confirmation of the original cytologic diagnoses was made in all cases.

Conclusions: Our study indicates that a specific diagnosis can be rendered from FNAB samples in the majority of cases and supports the diagnostic utility and accuracy of this technique in sampling primary bone lesions.

424 Value of p16/Ki67 Dual Immunostaining Evaluation of Cervical Cytology Specimen

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Background: Early diagnosis of cervical cancer is based on the detection of high-grade cervical intraepithelial neoplasia (CIN2-3). Although conventional cytology has a high diagnostic specificity has a poor sensitivity. On the other hand, molecular techniques of detection of hr-HPV markedly improve sensitivity but significantly reduce the specificity. It has recently been proposed that the use of p16/Ki67 dual immunostaining in cytological specimens could improve the accuracy of these tecniques. The main objective of this study is to assess the sensitivity and specificity of p16/Ki67 dual staining and compare it with the results of VPH detection using Hybrid Capture 2 (HC2).

Design: From October 2009 to July 2011, 527 women were included in the study (mean age: 35.8±10.9 years). All of them were referred to the colposcopy unit of the Hospital Clinic of Barcelona due to abnormal cytology results. Digital colposcopy, liquid-based cytology specimen (ThinPrep®), hr-HPV detection using HC2 (Qiagen) were obtained in all cases, and colposcopically directed biopsy and/or endocervcial curettage when clinically indicated. p16/ki67 dual immunostaining in cytology (CINtec plus, mtm) were performed in all cases. Definitive diagnosis was established after a complete study of the patients.

Results: After the completion of the study, 227 patients (43.1%) were classified as CIN2+, 153 (29.0%) as CIN1 y 147 (27.9%) as negative. A positive result for the dual immunostaining were detected in 91.6% of CIN2+, 51.6% of CIN1 and 6.8% of of the women with a negative study. A HC2 positive test was observed in 96.5% of CIN2+, 90.8% of CIN1 and 49.8% of the women with a negative study. The sensitivity, specificity, positive and negative predictive values for the identification of CIN2-3 of dual staining and HC2 are shown in table 1.

Table 1

	Sensitivity	Specificity	Positive predictive value	Negative predictive value
Hr-HPV (HC2)	96.5%	29.7%	50.9%	91.8%
P16/Ki67 dual staining	91.6%	70.3%	70.0%	91.7%

Conclusions: p16/Ki67 dual staining presents a sensitivity similar to HC2 with higher specificity values and could be used as complementary tool for conventional cytology and/or HC2 for diagnosis and follow-up of CIN2-3.

425 Should Cervical Cancer Screening Begin at Age 21? A Quantitative Analysis

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Background: In 2009 The American College of Obstetricians and Gynecologists recommended modifying the baseline cervical cancer screening age from the earlier of three years after first sexual intercourse or age 21 to age 21, regardless of the time of first sexual intercourse. In light of this recommendation, we evaluate whether Pap testing in women under the age of 21 in an urban high risk population group is necessary.

Design: The defined study group included women under the age of 21 who had their first abnormal cytologic diagnosis between 2001 and 2009. All subsequent cytologic and histologic diagnoses were recorded. Particular attention was taken of diagnoses occurring both before and after the subjects turned 21 years of age.

Results: A total of 2006 women aged 13 to 20 met the study criteria. Of these subjects, 92 (4.6%) were diagnosed with a high-grade squamous intraepithelial lesion (HSIL) prior to turning 21. Additionally, 1678 (84%) subjects had a cytologic diagnosis no greater than a low-grade abnormality [low-grade squamous intraepithelial lesion (LSIL) and atypical squamous cells of undetermined significance (ASCUS)] before turning 21, 13 of which developed HSIL after turning 21. Although not a specific category in the 2001 Bethesda System, 209 (10%) subjects were diagnosed, prior to turning 21 years of age, with a low-grade abnormality for which a higher grade lesion could not be completely excluded, 5 of which developed HSIL after turning 21.

A supporting histologic diagnosis, made prior to the subject turning 21, of CIN 2 or 3 was available for 32 subjects who had a diagnosis of HSIL prior to turning 21. A supporting histologic diagnosis, made after the subject turned 21, of CIN 2 or 3 was available for 7 subjects who were diagnosed with HSIL after turning 21, all of whom never received a diagnosis of either HSIL or CIN 2 or 3 prior to 21 years of age. Two subjects, both of whom had only a low-grade abnormality without a histologic diagnosis prior to turning 21, eventually developed invasive squamous cell carcinoma (21 and 25 years of age).

Conclusions: It appears that a substantial portion (15%) of abnormal cytologic diagnoses before the age of 21 are either HSIL or cannot completely exclude a high-grade lesion. Given the rate of progression of many of these lesions, our data suggests that active surveillance before the age of 21 in an urban high risk population group may yield a significant number of early diagnoses.

426 Cercariform Cells: Another Cytologic Feature Distinguishing Solid Pseudopaillary Tumor (SPPT) from Pancreatic Endocrine Neoplasms (PEN) and Acinar Cell Carcinomas (ACC) in Endoscopic Ultrasound-Guided Fine Needle Aspirates (EUS-FNA)

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Background: SPPT is a rare tumor of unknown origin occurring predominantly in the body or tail of the pancreas in young women. We have recently identified cercariform (Greek: tailed) cells, similar to those described in urothelial carcinomas in a EUS-FNA of a SPPT. We performed a multi-institutional study to define the value of these cells in the differential diagnosis of SPPT with other neoplasms characterized cytologically by the presence of monotonous uniform cells in pancreatic aspirates (PEN and ACC).

Design: The files of 2 academic institutions were searched for SPPT, PEN and ACC diagnosed by EUS-FNA. The slides were reviewed and a number of cytologic features recorded semiquantitatively in order to identify discriminating features between SPPT, PEN and ACC.

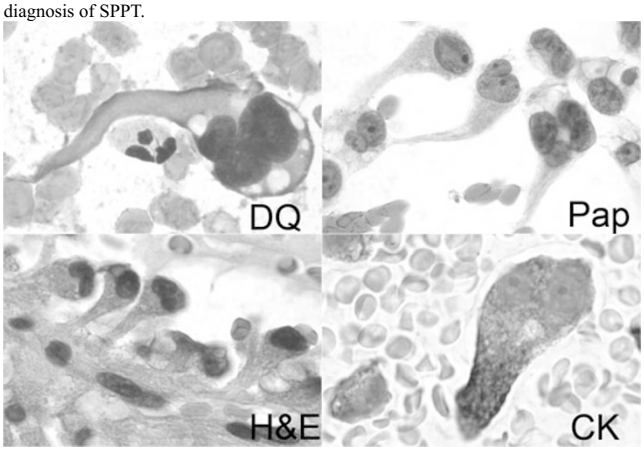
Results: 15 cases of SPPT, occurring in 14F/1M aged 22-64 (mean=39) were identified, together with 4 cases of ACC in patients aged 50-75 (mean=63), 3F/1M. 15 cases of PEN were randomly selected from the files of 2 institutions. They occurred in 5F/10M aged 35-79 (mean=56). The tumors were located in the pancreatic tail (SPPT n=8, PEN n=5, ACC n=0), body (SPPT n=4, PEN n=4, ACC n=0) and head of the pancreas (SPPT n=3, PEN n=6, ACC n=4).The mean size (in cm) of the tumors was: 4.7 for SPPT, 2.8 for PEN and 3.8 for ACC. The following features were common to SPPT, PEN and ACC: moderate to abundant cellularity, single cells, cell groups (rosettes/acinar formations), round to plasmacytoid cells, necrosis, pale to granular cytoplasm with fine vacuoles and bilobed/binucleated cells. Differentiating features of SPPT are shown in table 1.

Cytologic Features of SPPT, PEN and ACC

	Papillary structures	Cercariform cells	Large cytoplasmic vacuoles	Reniformnuc nuclei	Prominent nuclear grooves	Hyaline globules/ magenta-colored material	Degenerative features*
SPPT (n=15)	11	11	8	10	3	12	11
PEN (n=15)	1	1	1	0	0	0	0
ACC (n=4)	1	0	0	0	1	0	0

*Cholesterol crystals, calcifications, foam cells or giant cells

Conclusions: The presence of cercariform cells is a useful clue for the cytologic diagnosis of SPPT.



427 Osteoblastic Osteosarcoma: Cytomorphologic Characteristics and Differential Diagnosis on Fine Needle Aspiration

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Background: Osteoblastic osteosarcoma (OOS) is a uniformly fatal bone malignancy, if not diagnosed and treated appropriately in a timely manner. Fine needle aspiration (FNA) of osseous lesions is routinely performed in major medical centers. Appropriate characterization of the tumor will significantly influence the management and outcome.

Design: A retrospective search of the cytopathology archives of a large tertiary care center for a 22-year period (1989-2011) revealed 22 cases of OOS in 19 patients (primary-15, recurrent-5, metastatic-2). Material was obtained by FNA performed with radiologic assistance, except for one case for which the FNA was performed by the cytopathologist. Smears were stained with Diff Quik and Papanicolaou stains. Clinical outcome and histopathologic follow-up was reviewed and correlated.

Results: There were 11 males and 9 females (M:F, 4:3), ranging in age from 5-48 years (mean age 17.1 yrs). The anatomic locations were: distal femur (7), proximal humerus (2), proximal tibia (3), distal tibia (1), proximal fibula (1), iliac crest (2), pubis (1), sacrum (1), mandible (1) and soft tissue of thigh (3). All cases, except two, presented with progressively worsening localized pain, with or without swelling. The size of the lesions ranged between 1.8 cm to 34 cm (mean=8.9 cm). The initial FNA diagnoses were high grade malignant neoplasm (3), osteosarcoma, NOS (14) and OOS (5). Cytomorphologic characteristics were: variable cellularity, predominantly discohesive/single cells and small tissue fragments, focal osteoid, spindle to round plasmacytoid cells with moderate basophilic finely vacuolated cytoplasm, often with multiple cytoplasmic processes, round to oval nuclei with macronucleoli. 15/22 cases showed bi-nucleated and multi-nucleated osteoclast-like giant cells. Cases with high-grade histology displayed significant pleomorphism and abundant mitoses. Clinical outcome showed that eight patients had succumbed to the disease and 12 patients were alive at the time of this study.

Conclusions: An accurate FNA interpretation of OOS is significant, because immediate treatment is critical and the prognosis is better than other subtypes of osteosarcoma.
The differential diagnosis often includes reactive bone lesions and osteoblastoma.
The relative lack of significant pleomorphism and a larger population of plasmacytoid cells with fine cytoplasmic vacuolization are helpful distinguishing features.
A definitive diagnosis can often be made on FNA with clinical and radiological correlation.

428 Diagnostic Value of Thyroid Transcription Factor-1 and Thyroglobulin in Differentiating Thyroid Carcinoma and Adenocarcinoma of the Lung

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Background: Thyroid carcinomas and adenocarcinomas of the lung may share cytomorphological features while having vastly different clinical management. Occasionally, lung adenocarcinomas metastasizing to the thyroid or thyroid carcinomas metastasizing to the lung can be the initial presentation of these carcinomas. Thyroid Transcription Factor-1 (TTF-1) is routinely used to detect metastases of pulmonary adenocarcinomas and TTF-1 and Thyroglobulin (TGB) together are used to detect carcinomas of thyroid origin. We demonstrate the diagnostic value of TTF-1/TGB together to avoid misdiagnoses of thyroid carcinomas and lung adenocarcinomas.

Design: The cytopathology archives of a tertiary care center were retrospectively searched over a 10-year period (2001-2011) for cases of adenocarcinoma of lung versus thyroid. TTF-1 and TGB IHC were performed on all these cases. TTF-1 and TGB expression were reviewed and the data analyzed.

Results: Forty four cases of adenocarcinoma were found, that were worked up for adenocarcinoma of lung versus thyroid. The anatomic locations were: bone (4), liver (3), lung (5), lymph nodes (11-axillary-1, cervical-2, mediastinal-3, supraclavicular-3, subclavilar-1, not specified-1), neck (4), pleural fluid (8), soft tissue (4), and thyroid (4). Twenty six cases expressed TTF-1, but were negative for TGB. Twenty five of these cases were diagnosed as metastatic adenocarcinoma consistent with (c/w) a lung primary, two of which were in the thyroid and 1 case as adenocarcinoma of lung. Sixteen cases expressed both TTF-1 and TGB and were diagnosed as metastatic thyroid malignancies. Twelve of these were metastatic papillary thyroid carcinoma (PTC), one PTC, one metastatic follicular carcinoma and one poorly differentiated thyroid carcinoma. Three of these were metastatic PTC to the lung. Three cases were diagnosed as metastatic PTC, PTC and anaplastic thyroid carcinoma, while expressing TGB and being negative for TTF-1.

Conclusions: The clinical management of metastatic lung adenocarcinoma and metastatic thyroid carcinoma differ vastly. Among the cases in our study, two were those of adenocarcinoma of lung metastatic to thyroid and 3 cases were metastatic PTC to lung. While TTF-1 can be expressed in both lung and thyroid malignancies, TGB can be used as a differential marker. Having a high index of suspicion for metastatic lesions in cases that appear to be straightforward cases of primary lung or thyroid carcinomas will prompt the pathologist to evaluate these lesions for the expression of both TTF-1 and TGB so as to prevent misdiagnoses.

429 Pathological Diagnoses in Cases of Indeterminate or Unknown Primary Submitted for Molecular Tumor Profiling

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Background: Conclusive determination of tissue-of-origin has significant therapeutic implications and presents a major diagnostic challenge. This case series examines the pathologic characterization and role of diagnostic immunohistochemistry (IHC) in malignant tumor samples submitted for molecular tumor profiling using the 92-gene cancer classifier, CancerTYPE ID (CTID).

Design: De-identified cases (N=815) submitted for CTID testing were retrospectively analyzed. Sample characteristics including morphology, tumor grade, IHCs, suspected primary site, and time to completion (TTC; based on dates of sample collection and pathologist sign-out) were abstracted from pathology reports. Pathologic diagnoses were categorized as unknown, single suspected primary, differential (≥2 suspected), or no primary site reported. CTID data included probabilities, % tests with reportable results and TTC.

Results: 754 cases had evaluable pathology reports (age 62 y; 49% male; 82% carcinoma/ adenocarcinoma; 46% excision, 40% core needle, 14% FNA/cell block/ other). Conventional pathologic work-up included a mean of 7 IHCs (range: 0-35) and a mean TTC of 7.7 d (range: 0-127). CK7 (75%), CK20 (70%), TTF1 (63%) were the most common IHCs. 55% underwent all 3. Pathology reports indicated that upon submission for molecular testing 20% of cases had a single suspected primary, 53% a differential diagnosis, 8% were unknown, and 20% had no specified primary site. Notably, although cases with >7 IHCs vs ≤7 IHCs had longer TTC (9.3 vs 6.4d, p=.0017), they were not associated with an increase in single suspected primaries or a reduction in unknown diagnoses. CTID predicted a primary site in 91% of cases (median probability: 78%, range: 23-96%; mean TTC: 5.1d), and yielded similar results across biopsy types (excision: 92%, core needle: 92%, FNA/cell block/other: 89%). CTID provided a prediction in 93% (n=59) of cases with a pathologic diagnosis of unknown. In differential cases (n=399), CTID resolved the pathologic diagnosis in 55% and provided a new primary in 36%.

Conclusions: This large retrospective analysis demonstrated current IHC practices are nonstandardized in protocol, interpretation and TTC and there is a significant need for additional analytical approaches to aid in resolving indeterminate primary and

differential diagnoses. CTID is an objective, standardized test with a high analytical success rate that may facilitate diagnosis by providing adjunctive molecular data in tumors with indeterminate or unknown origin.

430 Repeat Fine Needle Aspiration Biopsy in Patients with Cytologically Atypical Thyroid Lesions

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Background: The Bethesda System for Reporting Thyroid Cytopathology includes two categories for classification of indeterminate lesions, "Atypia of Undetermined Significance" (AUS) and "Follicular Lesion of Undetermined Significance" (FLUS). The recommended follow-up for these lesions is to re-biopsy in three to six months. However, data on repeat biopsy of patients with atypical diagnoses are scant, and the best management for patients with two consecutive atypical biopsies remains unclear.

Design: A search of the PathNet clinical database was performed to identify thyroid fine needle aspiration (FNA) specimens collected between January 2009 and July 2011. The cytologic, imaging, and clinical characteristics and histologic follow-up was collected for all cases with an AUS or FLUS diagnosis.

Results: The search yielded 1177 thyroid FNA accessions. Atypical diagnoses were rendered in 125 cases from 117 patients. The ratio of atypical diagnoses to malignant diagnoses was 2.6:1. Forty-six (39%) patients underwent excision following the first atypical diagnosis while 39 patients (33%) were followed-up with a repeat biopsy performed 1 week to 13 months after the first biopsy (average 5 months). In 15 of these patients, the second biopsy was called benign. Twenty-three patients had an abnormal result on the second biopsy. Six of these were given a more definitive diagnosis, with three called diagnostic of papillary carcinoma and three called suspicious for follicular neoplasm. Seventeen of the atypical cases had a second biopsy that was also called atypical. In general, the atypical cytologic features seen in the first and second specimens (i.e microfollicular architecture, nuclear atypia) were similar. Of the fourteen double atypical cases that were excised, none were malignant. Six demonstrated follicular adenomas and remaining cases were hyperplasias or Hashimoto's thyroiditis.

Conclusions: Re-biopsy in patients with an atypical diagnosis on an initial biopsy serves to better clarify risk of malignancy. Of the patients re-biopsied in our cohort, all who had malignant lesions received a more definitive diagnosis on second biopsy. None of our patients with two consecutive atypical diagnoses had a malignancy on excision. This suggests that patients with repeat atypical results may benefit from continued clinical follow-up rather than surgery.

431 "Dense Squamoid Cytoplasm" and "Cellular Swirls" on Fine Needle Aspiration Cytology: Useful Ancillary Findings in the Diagnosis of Papillary Thyroid Carcinoma

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Background: "Dense squamoid cytoplasm" in cases of papillary thyroid carcinoma is described in some cytology textbooks, and "cellular swirls" are a recently recognized diagnostic clue to papillary carcinoma; however, their diagnostic significance has not been fully evaluated.

Design: The subjects were 50 cases of aspiration biopsy specimens of papillary carcinoma of the thyroid, which were histologically confirmed. In addition, 15 cases of adenomatous goiter, 10 cases of follicular neoplasm, 8 cases of medullary carcinoma and 2 cases of undifferentiated carcinoma were selected as control cases.

Results: Dense squamoid cytoplasm was observed in 25 cases (50%) of the papillary carcinoma. However, only one case of undifferentiated carcinoma revealed dense squamoid cytoplasm among the control cases. Dense squamoid cytoplasm was found as a sheet-like pattern or mildly overlapping pattern. Dense squamoid cells were slightly bigger than the usual follicular epithelial cells. The cytoplasm was polygonal and dense, and it had a distinct cell boundary. In addition, anisonucleosis was remarkable, and the nucleus was two to three times bigger than that of normal follicular epithelial cells. On the other hand, cellular swirls were found in 12 cases (24%) of the papillary carcinoma cases. Oval nuclei were arranged evenly in a swirl-like pattern. Control cases did not show cellular swirls.

Conclusions: Dense squamoid cytoplasm and cellular swirls were found in 50% and 24% of the cases of papillary carcinoma of the thyroid, respectively. Although both findings are not frequently observed, they are easy to identify at low magnification; therefore, they can be used as ancillary findings and deserve to be given more attention.

432 Utility of Fine Needle Aspiration for c-MYC Interphase Fluorescence In-Situ Hybridization Analysis of Aggressive B-Cell Lymphomas

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Background: c-MYC translocation is characteristically seen in Burkitt lymphomas and in approximately 10% of diffuse large B-cell lymphomas, not otherwise specified (DLBCL). In this study we report our experience on performing interphase fluorescence in-situ hybridization (FISH) analysis for c-MYC rearrangement on fine needle aspirates (FNA) of Burkitt lymphoma and DLBCL.

Design: We retrieved from our files 17 superficial/image-guided FNA cases of Burkitt lymphoma and DLBCL that had concomitant interphase FISH analysis performed for c-MYC rearrangement. Smears and/or cytospins were reviewed for sample cellularity and correlated with the results of the interphase FISH analyses.

Results: Interphase FISH analysis provided an informative results in 14 (82%) of the 17 cases (See table below). Most of these cases showed moderate to high cellularity. Only one case was paucicellular, however, the atypical lymphoid cells were intact and preserved. Three cases (18%) were non-informative for c-MYC status. On review, all three cases were paucicellular. One of these cases showed mostly degenerated cells with only rare intact atypical lymphoid cells. The remaining two cases showed primarily blood and no diagnostic atypical lymphoid cells.

c-MYC interphase FISH

Cellularity	Informative		Non-informative
	Positive	Negative	
Moderate to high	4	9	0
Paucicellular	0	1	3

Conclusions: FNA is a minimally invasive technique that provides sufficient and good quality material for c-MYC interphase analysis of aggressive B-cell lymphomas.

433 Cytomorphologic Criteria for the Distinction of Pulmonary Adenocarcinoma and Squamous Cell Carcinoma

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Background: Accurate distinction of lung adenocarcinoma (ADC) and squamous cell carcinoma (SQCC) is essential for the selection of patients for novel targeted therapies. While the distinction between well-differentiated SQCC and ADC is usually straightforward, classification of moderately and poorly differentiated (M-PD) carcinomas can be a challenge. We tested 20 cytologic features for sensitivity/specificity in distinguishing histologically-proven M-PD ADC and SQCC, and assessed the causes of diagnostic difficulties.

Design: We selected 70 lung cytology samples with a subsequent resection diagnosis of M-PD ADC or SQCC. Of these, 40 cases had "diagnostic" cytomorphology, whereas 30 cases were "difficult" in that they required immunocytochemical stains or were misclassified. Scored features of SQCC included keratinization, keratin rings, intercellular bridges, ghost cells, cell streaming, sharp cell borders, spindle cells, dense cytoplasm, dark coarse chromatin, pearl-like cell arrangements, stratified cell groups, frayed group edges, and extensive necrosis; of ADC: three-dimensional (3-D) cell balls, flat honeycombs, picket-fences, fine chromatin, prominent nucleoli, eccentric nuclei, and vacuolated cytoplasm. The distribution of these features was compared in ADC vs SQCC and "diagnostic" vs "difficult" groups.

Results: All features, except dense cytoplasm, were differentially distributed in ADC vs SQCC (p=0.005 - p<0.0001). Only 3 features were entirely specific for SQCC: cytoplasmic keratinization, keratin rings, and intercellular bridges, but none of these features was highly sensitive (56%, 67%, and 36%, respectively). The only entirely specific feature for ADC was flat honeycombs, and this feature was also highly sensitive (94%). Features with specificity >90% were ghost cells for SQCC, and 3-D cell balls and picket fences for ADC. The features associated with diagnostic difficulties in ADC were cell streaming (p=0.002) and sharp cell borders (p=0.0134); and for SQCC: eccentric nuclei (p=0.009) and fine chromatin (p=0.0006).

Conclusions: Many cytomorphologic features widely regarded as diagnostic or highly characteristic for distinguishing ADC vs SQCC are not entirely specific and represent potential diagnostic pitfalls. The ADC-like morphology of M-PD SQCC is well known, and is confirmed here. We also identified "squamoid" features of some M-PD ADC as a cause of diagnostic difficulty. Recognition of these features of overlap should trigger a work-up with immunocytochemistry to determine the line of differentiation.

434 Indeterminate Thyroid Cytology Cases with *BRAF* Mutations – Underlying Cytologic, Molecular, and Pathologic Characteristics

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Background: Mutation in the *BRAF* gene is highly specific for papillary thyroid carcinoma (PTC) and has been found predominantly in classical and tall cell variant of PTC. Therefore, cytology specimens with *BRAF* mutation are expected to show typical cytologic features and diagnosed as PTC. However, the more significant value of *BRAF* mutational testing may be in its potential to contribute toward the diagnostic accuracy of indeterminate thyroid cytology cases. In this study, we investigated the characteristics of the indeterminate diagnoses with *BRAF* mutation.

Design: Cytology cases demonstrating *BRAF* mutation in PTCs were selected from our pathology files from April 2007 to July 2011. From this group, we identified cases with the diagnoses of Atypia of Undetermined Significance (AUS), Follicular Neoplasm (FN), and Suspicious for Malignancy (SM) according to the Bethesda System. Samples were collected prospectively for cytologic analysis and molecular studies (placed into nucleic acid preservative solution). *BRAF* mutational analyses were performed by the real time polymerase chain reaction (PCR) and post-PCR melting curve analysis. The indeterminate diagnoses were correlated with the cytologic features, *BRAF* mutational status, and surgical pathology outcome.

Results: One-hundred twenty-one (121) cases of *BRAF* mutated PTC were identified. Of these, 33 cases (27%) were associated with indeterminate diagnoses. The correlations were summarized in the table. The *BRAF* K601E mutation was present in 9 of 33 indeterminate cases, but none in the SM group. There were no tall cell variant PTC cases and the vast majority of classic PTCs were in the SM group.

Correlation of the indeterminate diagnoses

Indeterminate Diagnosis	Cytologic Features	BRAF mutational status	Surgical Pathology Outcome of PTC type
AUS (n=16)	-mild atypia (9), hypocellular with focal atypia (5), focal microfollicles (2)	V600E (10), K601E (6)	MC (5), FV (3), Solid (1), CL (1), NOS (1), No F/U (5)
FN (n=4)	-microfollicular pattern (4)	V600E (1), K601E (3)	FV (3), MC (1)
SM (n=13)	-significant atypia (13)	V600E (13), K601E (0)	CL (8), MC (2), Warthin-like (1), NOS (1), No F/U (1)

NOS, not otherwise specified; MC, microscopic; FV, follicular variant; CL, classic; F/U, follow-up

Conclusions: The results of our study add validity to the classification scheme of the indeterminate diagnoses of the Bethesda System. The AUS, FN, and SM diagnoses in the setting of *BRAF* mutation are not only distinct cytologically, but appear to reflect differences in the molecular and surgical pathology outcome.

435 Fluorescent In-Situ Hybridization as an Ancillary Test for Residual Biliary Brush Cytology Specimens

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Background: The use of fluorescence in situ hybridization, specifically the Urovysion® probe kit, as an adjunct to routine evaluation of bile duct brushing specimens (BDB) has been demonstrated to be valuable in establishing a diagnosis of malignancy. To evaluate the performance of Urovysion® FISH on residual BDB, we collected material from 32 patient samples for which a cytologic diagnosis had already been rendered and compared the performance of FISH to cytology.

Design: Material remaining after standard cytology preparation was collected from 28 BDB samples stored in PreservCyt® or CytoLyt®. Slides were prepared manually and de-identified. Residual material from three FNA specimens and one pleural fluid specimen was prepared similarly. UroVysion® FISH was performed as described in the UroVysion® package insert. UroVysion® probes detect four chromosomal aberrations by FISH, including polysomy for chromosomes 3, 7, and 17, and the homozygous deletion of 9p21. UroVysion® FISH-stained slides were scanned using BioView Duet™ and re-classified by a cytotechnologist and a pathologist, who were blinded to cytologic outcomes.

Results: Of the 28 BDB specimens, 7 were positive for polysomy (7/28), 2 showed isolated trisomy of chromosome 7 (2/28), 6 were unsatisfactory (6/28) and 13 were negative (13/28). Of the 7 FISH-positive cases, cytology results were positive for adenocarcinoma in 4 cases, atypical/suspicious in 2 cases and negative in one case. The 2 trisomy cases by FISH were both atypical/suspicious by cytology. Only one of the 13 negative FISH cases was positive by cytology. Negative FISH results were found in 5 cases that had atypical cytology and 2 that were suspicious by cytology. Five cases were negative by both FISH and cytology. Four additional non-BDB cases were evaluated, including 3 FNA samples and one pleural fluid sample. One case showed atypical cytology and showed polysomy by FISH. Of the remaining 3 cases, all were adenocarcinoma by cytology and 2 were positive for polysomy by FISH.

Conclusions: Residual material from BDB specimens processed for cytologic diagnosis can be used for ancillary testing to support or exclude a diagnosis of malignancy, increasing the utility of these limited quantity samples. This may be particularly useful where cytology is indeterminate. The significance of a positive FISH result and a negative cytology result should be further investigated.

436 Does Mitosis Specific Marker PHH3 Help Grading Upper Tract Urothelial Carcinomas in Cell Blocks?

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Background: Grading upper tract urothelial carcinomas in cell blocks can be challenging. The interobserver agreement is poor among pathologists. Mitotic figure (MF) counting along with nuclear features is important in grading these tumors, however, artifacts, presence of apoptosis and rarity of MFs in a given lesion can hamper the task. We evaluated the use of the mitotic specific marker phospho-histone H3 (PHH3) as an adjunct to H&E stain for grading upper tract urothelial carcinomas in cell blocks.

Design: Formalin fixed paraffin embedded tissue from cell blocks of 61 urothelial carcinomas were stained with H&E and PHH3-antibody. Grading of tumors was performed by three pathologists in a blind fashion, first on H&E and then on both H&E and PHH3 stained slides. The grading system used was the 1973 WHO three point grading system. Gradings were compared across pathologists and for H&E staining versus PHH3 plus H&E staining with the Stuart-Maxwell test of marginal homogeneity that accounts for the matched data. The percent agreement and the kappa statistic were used to assess agreement. Statistical analyses were conducted in SAS 9.2 (SAS Institute Inc., Cary, NC) and Stata 11.2 (StataCorp, College Station, TX).

Results: Table 1 summarizes the inter-rater agreement between the three study pathologists.

Inter-Rater Agreement Among Pathologists Using H&E or PHH3+H&E Staining

	H&E	PHH3+H&E	P*
Percent Agreement (95% CI)			
I/II	56% (43%, 68%)	84% (74%, 93%)	0.002
II/III	48% (35%, 60%)	75% (65%, 86%)	0.003
I/III	61% (48%, 73%)	82% (72%, 92%)	0.016
kAPPA (95% CI)			
I/II	0.32 (0.16, 049)	0.74 (0.56, 0.92)	
II/III	0.21 (0.05, 0.37)	0.61 (0.42, 0.79)	
I/III	0.36 (0.18, 0.55)	0.71 (0.53, 0.89)	

I=First Pathologist; II=Second Pathologist; III=Third Pathologist. (*) P-value compares the percent agreement by H&E alone to that by PHH3+H&E

Conclusions: By adding PHH3 immunostain to the H&E, the agreement in grading the carcinomas among the three pathologists improved dramatically (Table 1, average pairwise agreement = 80%, overall kappa = 0.69). PHH3 immunostain may play an important role in grading upper tract urothelial carcinomas in small cell block samples.

437 Lymphoproliferative Disorders of the Kidney on Fine Needle Aspiration: A Study of 34 Cases

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Background: While extranodal spread of non-Hodgkin lymphoma (NHL) frequently affects the kidneys, primary renal lymphoma is rare. Making the diagnosis can be challenging in limited cytologic specimens. We review our experience with lymphoproliferative disorders in the kidney diagnosed by fine needle aspiration (FNA), with a focus on clinico-pathologic and radiographic features.

Design: All cases of NHLs diagnosed on renal FNA at two large academic institutions from 1989 until 2011 were reviewed. Demographics, clinical history, radiographic and cytomorphologic features, and follow-up were assessed.

Results: 34 cases were identified, 32 of which had available cytologic material for review. There were 16 primary tumors, 15 recurrences/secondary tumors, 2 post-transplant lymphoproliferative disorders, and 1 acute lymphoblastic lymphoma (ALL). There were 24 males and 10 females with an age range of 4.6 to 86.4 years (average 54.4 years). All lesions were of B-cell origin; the majority were aggressive/high grade (26/34). 25 of 34 cases were substantiated by positive flow cytometry results. The most common presenting symptom was pain, although the majority of lesions were detected at follow-up/incidentally. 21 cases presented as a solitary renal mass; the remaining cases showed multiple renal and or retroperitoneal masses. Salient radiologic features included hypodense, infiltrative, and ill-defined masses. Cytomorphologic characteristics included a monotonous population of large and atypical lymphoid cells, often with lymphoglandular bodies. Flow cytometry either displayed an abnormal population with clonal light chain expression or expression of blast markers (ALL case). Follow-up was available for 27 cases; 15 patients died of disease with a mean survival of 3.0 years (range 1 month to 8 years).

Conclusions: Renal lymphoma is an aggressive disease of older adults that is usually high grade. Although recurrences and/or systemic spread of lymphoma to the kidneys is far more common that primary tumors, involvement in these cases may not be documented by biopsy. Cytologic diagnosis of renal lymphoma requires analysis of morphological, clinical and immunophenotypic information. Certain helpful features for diagnosis include: flank pain and/or acute renal failure in an older patient, prior history of lymphoma, multiple homogeneous renal masses on CT, a monotonous population of large abnormal cells in a background of lymphoglandular bodies, and immunophenotyping demonstrating light chain restriction.

438 Effective Application of the Cellient™ Automated Cell Block Processor with Immunocytochemistry and Molecular Biology in Oncopathology

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Background: Immunocytochemistry and molecular biologic techniques performed on cell block material provide useful additional clinical information in cytopathology and oncopathology. A major factor determining the efficacy of a cell block technique is fixation of cell material. Most often formalin is used for cell fixation and only few data are available on the use of methanol-based solutions, e.g. those routinely used with the ThinPrep technique.

Design: We tested the accuracy of immunocytochemistry (ICC) and molecular diagnostic methods (ISH and PCR), using cell blocks made with the automated Cellient processor after methanol-based (PreservCyt) fixation of body cavity fluids and FNA material, applying 29 different antibodies. The quality of DNA and RNA after methanol fixation was tested with in situ hybridization using a SYT gene break-apart assay and EBER probes, as well as PCR using primer sets resulting in products of 100, 200, 300, 400, 500, and 600 bp. Moreover, we estimated the additional oncodiagnostic value of immunocytochemistry on Cellient cell blocks in a cohort of 100 consecutive cytology cases. For each case, the following items were scored: 1. adequacy of the specimen in terms of cellularity; 2. presence of benign or malignant tumor cells; 3. possibility to make a definitive diagnosis of a major tumor type (benign tumor, carcinoma, melanoma, lymphoma, others); 4. possibility to subtype carcinoma (adenocarcinoma, squamous cell carcinoma, neuroendocrine carcinoma); 5. in case of metastatic adenocarcinoma and small cell neuroendocrine carcinoma: possibility to determine the primary tumor location (lung, breast, thyroid, prostate); 6. determination of biomarkers relevant to tumor therapy (CD117 for GIST, ER and Her2/neu for breast carcinoma).

Results: Of 29 antibodies, 23 showed consisted ICC results on at least three consecutive samples. Distinctive and strong hybridization signals were observed for SYT and EBER. PCR products of linearly ascending bp size were readily observed with gel electrophoresis.

In our cohort of 100 consecutive cytology cases, additional and relevant diagnostic information was provided by ICC in 28 cases, applying diagnostic algorithms for sensitive ICC with this cell block technique.

Conclusions: Methanol fixation with the Cellient cell block technique allows for sensitive and specific immunocytochemistry and molecular biologic analysis. The latter will become increasingly important for the determination of patient tailored treatment in selected cancer types in the near future.

439 Diagnostic Value of BRAF (V600E) Mutation Analysis in Thyroid Fine Needle Aspiration Specimens in Indeterminate, Suspicious and Diagnostic Papillary Carcinoma. Our Institutional Experience in 45 Cases

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Background: Papillary thyroid carcinoma (PTC) is the most common cancer. The BRAF gene mutation is present in a significant number of cases of PTC and is predictive of some clinicopathological characteristics. Fine needle aspiration biopsy (FNAB) combined with molecular analysis provides great diagnostic accuracy and allows for selection for therapeutic decisions.

Design: Analysis of mutation status of BRAF (V600E) in samples from FNAB in the categories of indeterminate atypia, suspicious and diagnostic PTC.

BRAF (V600E) analysis was performed on FNAB material in 45 cases. 25 cases from retrospective follow-up patients and 20 prospective study (36 women / 9 men). Cytological diagnosis was: PC in 27/45 cases; suspicious for PC 8/45 cases; indeterminate 7/45 and benign 3/45 cases. Cytological diagnosis was confirmed by histology in 42 patients: 6 benign nodules and 32 classical variant of PC, 4 follicular variant. DNA was extracted and BRAF (V600E) mutation was detected by polymerase chain reaction followed by restriction enzyme digestion and sequencing of exon 15.

Results: BRAF (V600E) mutation was detected in 21 cases (46.6%), in 15 /21 with coincident previous diagnosis on cytology of PC and subsequent histology. 6 /21 cases were suspicious for malignancy in cytology and surgery was recommended. Definitive histological diagnosis of PC was established in all cases.Wild-type BRAF was detected in 24 cases (53.3%). No correlation was found in 12 cases (50%) with cytological and histological diagnoses of PC and wild-type BRAF, 4 patients had follicular variant of PC and in 3 of suspicious for malignancy and confirmed PC by histology. 2 cases of indeterminate atypia on cytology and wild –type BRAF had a benign final tissue diagnosis and 3 had PC. The absence of mutation was coincident with cytological diagnosis of benign nodules in 4 patients.

Conclusions: Prevalence of BRAF (V600E) mutation detected from FNAB specimens is 46.6% and all cases correlate with previous cytological and final histological diagnoses of PC. Wild–type BRAF is present in 53.3%, mutation was not detected in 4 cases of follicular variant. In problematical category of indeterminate atypia or suspicious, molecular analysis increase the accuracy of cytological evaluation and preoperative selection of patients.

440 Arginase 1 Is a Sensitive and Specific Marker for Distinguishing Hepatocellular Carcinoma from Metastatic Tumor

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Background: Distinction of liver metastatic tumor from hepatocellular carcinoma (HCC) may present a diagnostic challenge, especially in small tissue biopsy or fine needle aspiration (FNA) biopsy specimens. HepPar1 and glypican 3 are useful diagnostic markers, but the expression of these two markers has also been reported in non-hepatocellular tumors. Arginase 1 (ARG1) is a marker for HCC recently described in limited literature. In this study, we compared the expression of these three markers in HCCs and carcinomas from various organs on both surgical and FNA specimens using a single immunostaining system (Dako).

Design: Immunohistochemical evaluation of the expression of ARG1, HepPar1 and glypican 3 was performed on 1,294 cases of carcinomas from various organs on tissue microarray (TMA) sections and 66 cases of liver FNA specimens on cellblocks (29 HCCs; 31 metastatic tumors; and benign liver 6 cases). The staining intensity and distribution were recorded.

Results: The staining results are summarized in Table 1 and Table 2. For the non-hepatocellular tumors (N=1,276), 34 cases (2.7%) and 35 cases (2.7%) were positive for HepPar1 and glypican 3, respectively; in contrast, none was positive for ARG1. For FNA specimens, 20 HCC cases were positive for all three markers, and 8 HCC cases were positive for two markers. Only 1 case was negative for all three markers.

Table 1. Summary of immunostaining results on 1,294 surgical specimens

Diagnosis	ARG1 (positive cases, %)	HepPar 1 (positive cases, %)	Glypican 3 (positive case, %)
HCC (N=18)	17 (94%)	17 (94%)	14 (78%)
Lung AD (N=110)	0	9 (8%)	4 (3.6%)
Lung SCC (N=49)	0	0	9 (18%)
Breast CA (N=171)	0	0	4 (2.3%)
Esophageal CA (N=30)	0	8 (27%)	2 (7%)
Colon CA (N=71)	0	5 (7%)	0
RCC (N=132)	0	0	3 (2.3%)
Pancreas AD (N=50)	0	1 (2%)	0
Prostate AD (N=133)	0	3 (2.3%)	4 (3%)
Endometrial AD (N=38)	0	0	3 (8%)
Neuroendocrine CA (N=76)	0	0	0
Other organs (N= 416)	0	8 (1.9%)	6 (1.4%)

AD–adenocarcinoma; CA-carcinoma

Table 2. Summary of immunostaining results on 66 FNA specimens

Diagnosis	ARG1 (positive cases, %)	HepPar 1 (positive cases, %)	Glypican 3 (positive cases, %)
HCC (N=29)	23 (79%)	24 (83%)	24 (83%)
Metastasis (N=31)	0	0	3 (9.7%)
Benign liver (N=6)	6 (100%)	6 (100%)	1 (17%)

Conclusions: These data demonstrate that Arginase 1 has a similar sensitivity and higher specificity in differentiating a non-hepatocellular carcinoma from HCC when compared to HepPar1 and glypican 3. It is recommended to use three markers as a panel in distinction of HCC from metastatic carcinoma.

441 Role of Fine-Needle Aspiration Biopsy and Imaging in the Preoperative Workup of Salivary Gland Mass Lesions

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Background: Although not without controversy, fine-needle aspiration (FNA) and imaging are commonly used in the preoperative assessment of salivary gland mass lesions. Interestingly, few studies have examined the relative clinical value of these two assessment modalities. The goal of this retrospective study was to clarify the role of FNA and imaging in the workup of salivary gland masses.

Design: A computer-based search identified all patients with FNA of a salivary gland lesion during a ten-year study period. Only patients who had subsequent excision of their tumor with histologic diagnosis were included in the study. Chart review of all study patients was performed and information on lesion site, age, gender, radiologic diagnosis, pain in the tumor area, and facial paralysis was recorded and analyzed.

Results: 543 patients had FNA and subsequent histopathology. The majority of the tumors were in the parotid gland (n=492; 90.9%), followed by submandibular gland (n=45; 8.3%). The incidence of malignancy across all sites was 29.5%. The mean age was 54.1 years; 54.1% being female. The sensitivity, specificity, and diagnostic accuracy rates for FNA were 84.4%, 99.2%, and 94.8%, respectively. 464 patients had available radiologic studies. The sensitivity, specificity and diagnostic accuracy rates for imaging were 80.7%, 67.3%, and 71.7%. Older age, facial pain, and facial paralysis were independent predictors for malignancy (p-values <0.001, 0.006, and <0.001 by univariate analysis, respectively; odds ratio 1.470, 1.960, and 14.674 by multivariate analysis, respectively.)

Conclusions: Contrary to previous reports, FNA is more reliable than radiologic imaging in evaluating benign and malignant salivary gland lesions and is helpful in surgical planning and counseling. Routine imaging may not be necessary and may be most appropriately suited for anatomic definition of the lesion and assessing for metastatic disease in selected cases. We emphasize:

1. FNA is a reliable tool in the preoperative workup of both benign and malignant salivary gland lesions.
2. Preoperative imaging has a lower sensitivity and specificity than FNA and is not as accurate at differentiating benign from malignant lesions.
3. Older age, facial pain and facial paralysis are independently associated with malignancy.

442 Follicular Variant of Papillary Thyroid Carcinoma: Accuracy of FNA Diagnosis and Implications for Patient Management

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Background: Follicular Variant of Papillary thyroid carcinoma (FVPTC) has created a continuous diagnostic dilemma among pathologists because of the paucity of nuclear changes of papillary carcinoma and overlapping features with benign and other neoplastic follicular lesions. Current guidelines for the management of thyroid nodules recommend surgery for confirmed PTC, suspicious and follicular neoplasm cases, while further immediate diagnostic studies or treatment are not routinely required if the nodule is benign on cytology. This study is designed to determine the accuracy of cytology in the diagnosis of FVPTC, based on the Bethesda classification system and determine the implications for patient management based on the current recommendation.

Design: Based on a retrospective review of cytologic diagnosis between January 2008 and December 2011, thyroid FNA cytology specimens with subsequent surgical intervention and a final diagnosis of FVPTC were selected from our files. The cytologic diagnoses were compared with the final diagnoses and the percentage of cases contributing to the final diagnosis of FVPTC was calculated for each diagnostic category. Triage efficiency and diagnostic accuracy were calculated.

Results: One hundred and fifty two cases with histologic confirmation of FVPTC were identified (representing 128 patients – 100 females, 28 males). All patients had undergone either lobectomy with completion thyroidectomy or total thyroidectomy. The cytologic diagnosis of "positive for malignancy" accounted for only 26% of the final histologic diagnosis of FVPTC while suspicious for carcinoma, follicular neoplasm, follicular lesion of undetermined significance and benign accounted for 11%, 22%, 22%, 15% of the final diagnosis of FVPTC, respectively. Non-diagnostic cytologic cases accounted for the remaining 4%. Only 18% of the 55 cases tested were positive for BRAF mutation.

Conclusions: The subtle nuclear features of FVPTC pose challenges for an accurate diagnosis. Therefore a better approach is to triage these cases for surgical intervention and/or further evaluation of the particular nodule. Our triage efficacy for FVPTC was 84%, however the diagnostic accuracy of PTC was 38%. Up to 15% of cases may have no further immediate diagnostic studies or treatment. BRAF mutation analysis has no statistical significance on diagnostic accuracy.

443 Follicular Neoplasm: Evaluation of the Risk of Malignancy Using the Modified Bethesda Classification

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Background: The Bethesda 2007 Thyroid Cytology Classification defines Follicular Neoplasm as a category of cases with cellular specimens showing abundant follicular cells arranged in a microfollicular pattern with little or no colloid. The current recommendation for the management of these cases is lobectomy. There has been great difficulty and variability in triaging and reporting follicular neoplasm. In our institution, this category is further subclassified into two: 1) Microfollicular patterned neoplasm (FN1) and 2) Follicular lesion with some features suggestive of but not diagnostic of follicular variant of papillary thyroid carcinoma (FN2). We reviewed the cases of follicular neoplasm seen over a period of three years to document the follow-up trend using this two-tier classification.

Design: A search of the cytology records was performed for the period between January 2008 and December 2010. All thyroid FNA cases were reviewed and the ones with the diagnosis of follicular neoplasm (including Hurthle cell neoplasm) were identified. Correlating follow-up surgical pathology reports were reviewed. The percentage of cases showing a malignancy was calculated.

Results: Two hundred and forty six cases of follicular neoplasm with surgical follow-up were identified (217 FN1 and 29 FN2). Malignancy was identified in 32% of all FN cases. This was disproportionately higher in the FN2 (72%) compared to the FN1 (27%) cases. In the FN1 category, malignancy rate for Hurthle cell neoplasm and FN, NOS were 28% and 26%, respectively. The malignant cases were largely follicular variant of papillary carcinoma. When the benign cases were further classified into neoplastic vs. non-neoplastic, 57% were neoplastic and were predominantly cases of follicular adenoma and Hurthle cell adenoma, while 43% were non-neoplastic and were predominantly nodular goiter.

Conclusions: The FN2 category requires a more aggressive follow-up than the FN1 category and justifies an immediate referral for lobectomy. The FN1 category may require further triage using other ancillary methods. The rate of malignancy in the present study is 32%, a higher end of previously reported values in the literature.

444 Cytomorphology, Cyst Fluid Analysis and Molecular Tests in Pancreatic Cystic Lesions: Review of 459 Cases

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Background: Endoscopic ultrasound-guided fine needle aspiration (EUS-FNA) is increasingly used to evaluate cystic lesions of the pancreas. It is however quite difficult to accurately diagnose these lesions due to scant cellularity and subtle cytomorphologic features. Cyst fluid analysis and molecular tests may help improve diagnostic performance. In this study, we retrospectively reviewed our experience with cystic pancreatic lesions diagnosed by EUS-FNA.

Design: The electronic data of cytopathology archives were searched for pancreatic lesions diagnosed by EUS-FNA at our institution during the period from January 2005 to June 2011. A total of 1,143 cases were retrieved, of which 459 cases (40%) were cystic lesions. The cytomorphologic diagnoses and the results of cyst fluid analysis (amylase and CEA levels) and molecular tests (K-ras mutation, loss of heterozygosity [LOH] of tumor suppressor gene alleles) were reviewed. Histopathologic follow-up was available for comparison in 81 cases.

Results: The cytomorphologic diagnoses included non-diagnostic (48 cases, 10%), negative (127 cases, 28%), bland/cyst contents (160 cases, 35%), atypical (26 cases, 6%), pancreatic endocrine neoplasm (6 cases, 1%), mucinous cystic neoplasm (84 cases, 18%), and malignant (8 cases, 2%). Elevated CEA level (>=192 ng/ml), positive K-ras mutation and LOH were found in 25 of 79 (32%), 49 of 137 (36%), and 54 of 94 (53%) cases, respectively. Histopathologic follow-up was compared with cytomorphologic diagnoses (Table 1) as well as the results of cyst fluid analysis, K-ras mutation and LOH test (Table 2).

Table 1. Correlation between Histopathologic and Cytopathologic Diagnoses

	Cytological Diagnosis						
Histopathology	Non-diagnostic	Negative	Bland/Cyst	Atypical	PEN	MCN	Malignant
Negative (13)	0	3	8	0	0	2	0
PEN (6)	1	0	0	0	5	0	0
IPMN (31)	2	3	7	4	0	11	4
MCN (28)	1	8	12	1	0	6	0
Malignant (3)	1	0	0	0	0	0	2
Total (81)	5	14	27	5	5	19	6

PEN, pancreatic endocrine neoplasm; IPMN, intraductal papillary mucinous neoplasm; MCN, mucinous cystic neoplasm

Table 2. Correlation between Histopathologic Diagnosis and Ancillary Studies

	CEA		K-ras Mutation		LOH	
Histopathology	< 192 ng/ml	>=192 ng/ml	Negative	Positive	Negative	Positive
Negative	1	0	1	1	1	0
PEN	2	0	2	0	1	0
IPMN	1	3	8	6	3	5
MCN	0	7	8	8	3	8
Malignant	0	1	1	0	1	0
Total	4	11	20	15	9	13

LOH, loss of heterozygosity **Conclusions:** Our data demonstrate that cyst fluid analysis and molecular tests are complementary to cytomorphologic evaluation and should be incorporated in the final cytological diagnosis of pancreatic cystic lesions.

445 Improving the Predictability of Indeterminate Results of Urinary Cytologic Samples: An Outcomes and Cytomorphologic Study

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Background: In most cytopathology laboratories, urinary tract (UT) samples are second only to Pap tests in annual volume. We previously designed a template in order to standardize our UT diagnostic categories to enable our clinicians to uniformly manage their patients. We have also examined the common cytomorphological features found in the category "atypical urothelial cells, suspicious for high grade urinary carcinoma (AUC-H)" that proved most predictive of high-grade urothelial carcinoma (HGUC). These features are utilized in the current study, a training exercise, in an attempt to improve the predictive value of this clinically frustrating category.

Design: The hospital laboratory information system was searched for cytology specimens that were diagnosed as "Atypical urothelial cells of undetermined significance" (AUC-US) from July 1, 2007 to June 30, 2009. 160 specimens from 118 patients were identified and classified by clinical indication (surveillance for neoplasia or hematuria). 123 specimens with subsequent biopsy or longitudinal follow-up were selected for preliminary review. A junior and senior pathologist, blinded to outcome, separately evaluated each of the AUC-US specimens for individual cytologic criteria found to be most predictive of HGUC in specimens diagnosed as AUC-H. The predictions were then matched with the follow-up biopsy or clinical outcomes, which were tracked over the 18 months following the July 2009 cutoff for inclusion in the study.

Results: For surveillance patients (n = 67), 48% had benign follow-up, 17% had LGUC, and 30% had HGUC. The sensitivity and specificity of the criteria in these patients were 86% and 59%. For patients with hematuria (n = 56), 11% were diagnosed with nephrolithiasis, and 10% were diagnosed with HGUC; the remainder of the samples were considered benign. In this patient group, the sensitivity and specificity of the criteria (57% and 41%) were both poor.

Conclusions: Patients with urine specimens classified as AUC-US at our institution are less likely to be subsequently diagnosed with HGUC. We have defined cytologic criteria which predict the risk of HGUC on follow-up with a high sensitivity (86%) and acceptable specificity (59%) in surveillance patients. These criteria from our "training set" will be further refined following the examination of our remaining AUCUS specimens, which will serve as our "test set". Reclassifying specimens meeting these criteria into a category with a higher level of suspicion for HGUC (ASC-H) may help guide clinicians to provide more appropriate follow-up to these patients.

446 Urine Cytology for Investigation of Primary Hematuria: A Redundant Test?

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Background: It is standard practice to use urine cytology in conjunction with flexible cystoscopy (FC) and radiology to investigate patients with hematuria. We evaluated the clinical utility of urine cytology in this setting.

Design: 1360 consecutive patients with primary hematuria underwent urine cytology, FC and ultrasound scan (USS) of the urinary tracts in rapid diagnosis hematuria clinics in two hospitals during a 1 year period. The electronic notes of these patients were reviewed for the results of these investigations and for follow-up information during a period ranging from 22-34 months. Urine cytology results were classified as U1: unsatisfactory; U2: negative for malignancy; U3: atypia uncertain significance; U4: suspicious for malignancy and U5: malignant. U4 and U5 urine cytology diagnoses were considered positive as patients with U4-5 cytology and negative initial histology would be subjected to further investigations such as rigid cystoscopy, random bladder biopsies, CT urography and ureteroscopy. The positive end point was a histological diagnosis of malignancy.

Results: The distribution of cytological diagnosis and the frequency of malignancy (bladder, kidney or prostate) on follow-up are shown in Table 1.

Table 1

Diagnostic category	Number of cases (%)	Number of cases with cancer on follow-up
U1	16 (1.2)	0 (0)
U2	977 (71.8)	36 (3.7)
U3	277 (20.4)	52 (18.8)
U4	56 (4.1)	10 (71.4)
U5	34 (2.5)	31 (91.2)

The prostate cancers were mass lesions involving the bladder. The sensitivity, specificity, negative predictive value, positive predictive value, false positive rate and accuracy of urine cytology was 44.9%, 98.4%, 93.2%, 78.9%, 1.4% and 92.2% respectively. 69/71 (97.2%) patients with true positive urine cytology had abnormal FC or USS. In the other 2 cases FC had not been performed. Urine cytology did not pick up even a single case of cancer in which both FC and USS had shown no evidence of malignancy.

Conclusions: Urine cytology has no clinical utility in the investigation of hematuria if the patients are investigated with FC and USS. However urine cytology could still have a role in the surveillance of patients with urothelial carcinoma who would have a higher risk of urothelial carcinoma in situ that may not be identified by FC and USS.

447 Bile Duct Brushing Cytology Molecular Evaluation: Comparative Analysis of the Slide Based Cytology and Cell-Free Supernatant Fluid for Mutational Change

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Background: Bile duct brushing cytology plays a prominent role in confirming the presence of extrahepatic biliary tract malignancy. However, its value is limited by its relatively low sensitivity. Some of the factors that influence the accuracy of cytologic

diagnosis are attributed to specimen adequacy, inflammation and sampling variation. We explored an alternative approach that is not dependent upon slide based cellularity, but rather uses the cell-free supernatant fluid accrued during cytology processing.
Design: 8 cases underwent analysis (5 benign, 3 malignant). The bile duct brushes were placed in saline and fixative after preparation of direct smears. Cytospin smears and a corresponding cell-free supernatant fluid were prepared. Both types of specimens underwent molecular analysis with comparison of mutational profiles. DNA, extracted from 2 ml of the cytocentrifugation supernatant fluid, was quantified by optical density and amplified by qPCR. Mutational analysis followed using PCR/capillary electrophoresis for a broad panel of markers (KRAS point mutation by sequencing, microsatellite fragment analysis for loss of heterogeneity of 16 markers at 19, 3p, 5q, 9p, 10q, 17p, 17q, 21q, 22q). In selected cases, microdissection of stained cytology smears and/or cytospin smears were similarly analyzed and compared. Cytology and surgical pathology was used to define outcome status.
Results: 3 of 8 (37.5%) of the samples were hypocellular; however, DNA level (3.3-68.9 ng/ ul) was sufficient to be examined. DNA quantity was significantly higher in cases of malignancy (ave. 45.1 ng/ul) compared with non-neoplastic (ave. 5.5 ng/ml, p<.001) cases. No mutational change was present in the supernatant fluid of non-neoplastic specimens. All cases of malignancy demonstrated mutational change (3-8 mutations). Analysis of the supernatant fluid showed equal or additional mutations compared to that of the microdissected stained cytology smear.
Conclusions: 1. Mutational changes were detected in supernatant fluid of all malignant compared with benign cases. 2. The presence of mutated free DNA in the extracellular fluid rather than microdissected slide based cells specimen could serve as a valuable alternative to support a malignant diagnosis, especially when the specimen has limited cellularity.

448 Clinicopathological Significance of Perivascular Mesenchymal Cell Clusters in Imprint Cytology of Lymph Nodes

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Background: In stump cytology specimens of lymph nodes, other than lymphocytes, there are many mesenchymal cells. Particularly, the high endothelial venule (HEV) is well known. Furthermore, large mesenchymal cell clusters composed of smooth muscle actin-positive spindle cells and CD34-positive endothelial cells are observed in cytological specimens. We termed these spindle cells Perivascular Mesenchymal Cell Clusters (PVMCCs).
In the last meeting, we reported that PVMCCs were observed in 76% of T-cell lymphoma and 47% of Hodgkin lymphoma, but rarely in reactive lymphoid hyperplasia. Further, PVMCCs are very useful to differentiate between malignant lymphoma and reactive lymphoid hyperplasia.In this study, we report the clinicopathologic features of PVMCC-positive lymphomas.
Design: Between October 2008 and August 2010, cases involved 143 imprint specimens of lymph nodes, 65 cases of B-cell lymphoma, 16 cases of T-cell lymphoma, 17 case of Hodgkin lymphoma, 35 cases of reactive lymphoid hyperplasia, 5 cases of Castleman disease, and 5 cases of EBV-associated B cell lymphoproliferative disorder.
Two consecutive slides were prepared from each biopsy specimen. The slides were immediately fixed in 95% ethanol and stained using the standard Papanicolaou method. Clinicopathologic data were obtained from medical records. Cytogenetic analysis was performed in all cases.
Results: PVMCCs were found in 10 of the 65 cases of B-cell lymphoma, in 13 of the 16 cases of T-cell lymphoma, and 8 of the 17 cases of Hodgkin lymphoma. But PVMCCs were seen in only 2 of the 35 cases of reactive lymphoid hyperplasia. No PVMCCs was observed in the cases of Castleman disease.
The average soluble IL-2 receptor (sIL-2R) levels were 2,318 U/ml in PVMCC-negative cases and 6,393U/ml in PVMCC-positive ones (p<0.01). The median overall survival was 896 days in PVMCC-positive cases and 1074 days in negative cases, with a median follow-up of 1029 days (p=0.038).
Four of the 5 cases of EBV associated B cell lymphoproliferative disorder showed many PVMCCs, and a higher sIL-2R level (average: 3823U/ml), and 3 cases died (345, 435, 913 days after the diagnosis).
Conclusions: In the PVMCC-positive cases, sIL-2R levels were significantly higher than those of negative cases. The PVMCC-positive cases show a poor prognosis. The presence of PVMCCs suggested an unfavorable outcome.

449 SIRT-1 Over-Expression and Its Association with p16INK4a in Cervical Intraepithelial Lesions

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Background: Epigenetic modifications of proteins, histones, and chromatin play an important role in relating gene expression, cancer formation, and life span. Recent evidence indicated that epigenetic changes might 'addict' cancer cells to altered signaling during the early stages of tumor development. SIRT1 plays a significant role in epigenetic modifications and is significantly elevated in many cancers. HPV E7 has been shown to up-regulate SIRT1 levels in cervical cancer cell lines, but the expression of SIRT1 in cervical intraepithelial lesions (CIN) is unknown. P16INK4a has been shown to be a very sensitive surrogate marker for hrHPV infection in CIN, so we studied the correlation between SIRT1 and p16INK4a.
Design: 77 cases (58 cervical biopsies and 19 LEEP) were selected including 29 CIN1, 32 CIN2 and 16 CIN3. All H&E slides were reviewed and the CIN diagnoses were confirmed. SIRT1 and p16 IHC were performed, and the non-lesion tissue on LEEP specimens was served as the normal controls. The staining intensity and location of SIRT1 and p16 were correlated. Fisher exact test was used for the statistic analysis.
Results: Normal cervical tissue was negative for SIRT1 except for weak positive in the basal layer. Over-expression of SIRT1 was found in 13.8% CIN 1 (4/29), 40.6% CIN2 (13/32), and 50% CIN3 (8/16), and it was statistically significant between CIN1 and CIN2/3 lesions (p=0.01). Strong diffuse p16 positive was observed in 20.6% CIN1 (6/29), 81.2% CIN2 (26/32), and 100% CIN3 (16/16) (CIN1 vs CIN2/3, p=0.0001). 22/25 cases with over-expression of SIRT1 were correlated and colocalized with p16INK4a (88%). The 3 cases with over-expression SIRT-1 and negative p16 were 2 CIN1 and 1 CIN2 cases.
Conclusions: Over-expression of SIRT1 was observed in many CIN2/3 and few CIN1 lesions and it was correlated and colocalized with p16INK4a. This is the first study to show the up-regulation of SIRT1 in CIN, and the meaning of SIRT1 over-expression found only in some CIN 2/3 lesions is not clear so far. It is possible that SIRT1 over-expression may predict the disease progression. Future study including microinvasive and invasive cervical cancers may provide some useful information. Additionally, our data did support the current concept that p16 is a sensitive biomarker in the diagnosis of high-grade CIN.

450 Cytological Identification of Adenocarcinoma of the Lung with Minimal Use of Immunohistochemistry. Can We Meet the Challenge?

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Background: Adenocarcinoma is the most common histologic type of lung cancer. To address recent advances in therapy of lung adenocarcinoma IASLC/ATS/ERS has put forward a new adenocarcinoma classification. This requires a multidisciplinary and strategic management of tissue for molecular and immunohistochemical studies. For the first time, the new classification addresses an approach to small biopsies and cytology in lung cancer diagnosis. It has been recommended that the use of immunohistochemistry at the time of primary evaluation should be restricted to the use of a two stain panel and to preserve as much tissue as possible for potential molecular markers, thereby laying more emphasis on morphological categorization. Unfortunately, morphology often is bypassed in favor of immunohistochemistry in routine pathology. In this study we attempted to see how far we can correctly categorize non small cell carcinomas without the use of immunostains.
Design: 50 cases of cytologically diagnosed non small cell type lung carcinomas were blind reviewed by one of the author (VM) with an attempt to categorize them into adenocarcinoma and squamous cell carcinoma based on cytological features alone. The findings were compared with the initial diagnosis. Results of immunohistochemistry if used for the initial diagnosis was documented.
Results: On retrospective blind review, 26 cases were categorized into adenocarcinoma and 14 cases were categorized as squamous cell carcinoma. There were 10 cases that were called non small cell carcinoma. In comparison at the time of initial diagnosis, 24 cases were called non small cell carcinoma (including the 10 cases on blind review). In 24 cases immunohistochemistry was used for further subtyping at the time of initial diagnosis and use of 2 stains (p63 and TTF-1) amongst multiple stains, were helpful in a definitive categorization. Of the 10 non small cell carcinoma called at the time of initial diagnosis and at the time of blind review, 4 turned out to be squamous cell carcinoma. There were 2 cases that could not be categorized even on resection specimen; thereby highlighting that use of large panels of immunohistochemical stains does not provide an advantage over routine light microscopic diagnosis.
Conclusions: When attempted, at least 90% cases of adenocarcinomas can be identified based on cytological features alone and with use of a two stain immunostains panel. Poorly differentiated squamous cell carcinoma is more likely to be called a non small cell carcinoma.

451 Follow-Up Outcomes of a Large Cohort of Low-Risk Patients with Negative Imaged Liquid-Based Cytology and Negative HPV Test Results

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Background: Concurrent testing for HPV and cytology (co-testing) is an FDA-approved cervical screening alternative for women 30 years and older. The objective of this study was to document the development of significant cervical disease over time in a large cohort of low risk women with "double-negative" co-test results.
Design: The CoPath database was searched from July 2005 to June 2006 to retrieve cases with both negative hrHPV and cytology results. hrHPV testing used the HC2 assay. Pap tests were LBC ThinPrep specimens screened utilizing computer-assisted screening. Pap, histologic, and repeat HPV test follow-up results were recorded and analyzed.
Results: Double-negative cotest results were identified in 4112 women, and 3211 had recorded histopathologic and/or cytological follow-up results. The average age of the women was 46.8 years (15-87). The average follow-up period was 44 months (1-69). Histopathologic and cytologic follow-up results are shown in Table 1.

Summary of Follow-up Results

Follow-upMethod(s)	Total Case#	Glandular neoplasia*	CIN2/3/HSIL#	CIN1/LSIL	CIN/SIL
Histology	549	6	5 (0.9)	50 (9.1)	55 (10.0)
Cytology only	2662	0	1 (0.04)	24 (0.9)	25 (0.9)
Total	3211	6 (0.2)	6 (0.2)	74 (2.3)	80 (2.5)

*Includes diagnoses of one AIS, one invasive cervical adenocarcinoma, and four endometrial carcinomas. #One CIN3 with microinvasion

The average follow-up period for cases with CIN2/3 diagnoses was 36.6 months (11-64). The average follow-up period to an initial Pap test was 24 months (1-68). 2023 women with had repeat HPV testing and 107 (5.3%) had hrHPV-positive results.

Repeat HPV testing result

HPV testing	Case No	%
Positive only	56	2.8
Negative only	1916	94.7
Both positive and negative	51	2.5
Total	2023	100

An initial repeat hrHPV test was positive in 89 of 2023 (4.4%) patients. The average follow-up interval to an initial repeat hrHPV test was 31 months (1-69) and 34 months (5-69) to an initial repeat positive hrHPV test.

Conclusions: 3211 women who were negative by both cytology and HPV testing had histologic and/or Pap follow-up results over an average follow-up period of 44 months. Two patients were diagnosed with cervical carcinomas. High grade cervical intraepithelial neoplasia was diagnosed in seven patients. The risk of developing cervical high grade neoplasia for women with double negative co-testing results is very low. Our result also indicated that the "3-year screening intervals" guideline was not strictly followed in clinical practice for this study group.

452 Endometrial Wash Cytology Revisited Utilizing 101 Cases with Subsequent Endometrial Biopsies among Postmenopausal and Perimenopausal Women with Vaginal Bleeding

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Background: Postmenopausal/perimenopausal vaginal bleeding requires full evaluation of the endometrium including vaginal ultrasonography and pathological evaluation. Endometrial biopsy is commonly performed to exclude cancer and endometrial hyperplasia. However, the positive yield of endometrial biopsies is less than 100% because the abnormal endometrium may be focal, occupying less than 5% of the surface area of the endometrial cavity or less than 25% of the area in those confined to the endometrial polyp. The use of endometrial wash examination may add valuable pathological information since cells from different parts of the uterine cavity are sampled.

Design: To evaluate whether saline endometrial wash will yield additional valuable pathological findings a total of 101 cases of endometrial saline wash followed by endometrial biopsy among women (52 to 72 years old) with vaginal bleeding was reviewed.

Results: Of the 101 cases, ninety showed negative findings (negative for glandular atypia/dysplasia or carcinoma) in both endometrial wash and endometrial biopsy. There were eleven cases (10.9%) of endometrial wash with positive findings (presence of either glandular atypia/dysplasia or carcinoma). There were six cases diagnosed with adenocarcinoma in both endometrial wash and endometrial biopsy. Five of these cases underwent hysterectomy in our hospital and the hysterectomy specimens also showed adenocarcinoma. One case transferred to another institution and no follow-up information is available. There were five cases diagnosed with atypical glandular cells. The endometrial biopsies of these five cases showed: a.) one well differentiated adenocarcinoma, b.) two glandular atypia, c.) one endometrial hyperplasia with atypia, and d.) one negative. The last case with a negative biopsy finding, repeat endometrial wash and biopsy were performed. The repeat endometrial wash revealed adenocarcinoma, however, the endometrial biopsy was negative. The patient transfered to another institution were an endometrial biopsy was also performed which showed no evidence of malignancy. Following a review of our endometrial wash cytology material the patient underwent hysterectomy. The hysterectomy specimen revealed multiple endometrial polyps with in situ high grade serous carcinoma.

Conclusions: Our data therefore, although limited, showed that endometrial wash in addition to endometrial biopsy may offer valuable pathologic information in the work-up of women with perimenopausal and postmenopausal bleeding.

453 Pap Tests with Both Atypical Squamous Cells of Undetermined Significance and Infectious Organisms in Liquid-Based (Surepath) Pap Tests: Impact on the Prevalence of Human Papillomavirus Infections and Follow-Up Biopsy Diagnoses

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Background: Despite its use for more than two decades and the clearly formulated Bethesda system criteria, the diagnosis of atypical squamous cells of undetermined significance is still the most challenging and least reproducible diagnosis made on Pap tests. The diagnostic challenges can be exaggerated by the confounding effect of the reactive changes caused by infectious organisms (ORG). The significance of the association of ASC-US with infectious organisms has been controversial with some studies finding that the presence of ORG leads to an overcall of ASC-US, while others have shown an increased incidence of HPV detection in cases of ASC-US associated with ORG, especially Candida. The aim of this study was to determine the effect of infectious organisms (bacterial vaginosis (BV), Cadida (Cand) herpesvirus (HSV) and Trichomonas (TV)) on HPV rates and types in cases diagnosed as ASC-US on liquid-based (Surepath) Pap tests.

Design: We identified all cases diagnosed as ASC-US with concomitant infectious organisms (ASC-US+ORG) from 1/1/2003 to 12/31/2010 that had reflex HPV testing performed by a PCR-based method using MY09/11 consensus primers and typing by RFLP. The prevalence of any HPV type, high-risk HPV types (HR-HPV) and HPV16/18 and the frequency of abnormal follow-up biopsies diagnosed as CIN1 and above (CIN1+) and CIN2 and above (CIN2+) was compared to those of cases of ASC-US without associated organisms (ASC-US-ORG) statistically.

Results: Of the 23077 total ASC-US cases that fulfilled the study criteria, 2344(10.2%) were ASC-US+ORG (1015+BV, 1276+Cand, 23+HSV, 30+TV).

	AGE (MEAN ±SD)	ALL HPV TYPES	HR-HPV TYPES	HPV TYPES 16/18	BIOPSY RATE	CIN1+	CIN2+
ALL ASC-US (N=23077)	36.3±13.2	8937 (38.7%)	4277 (18.5%)	1993 (8.6%)	4241 (18.4%)	1845 (8%)	581 (13.7%)
ASC-US-ORG (N=20733)	37±13.25	7625 (36.8%)	3624 (17.5%)	1703 (8.2%)	3701 (17.9%)	1593 (7.7%)	493 (2.38%)
ASC-US+ORG (N=2344)	30±10.7	1312 (56%)	653 (27.9%)	290 (12.4%)	540 (23%)	252 (10.8%)	88 (3.75%)
ASC-US+ORG VS. ASC-US-ORG %CHANGE		+52.2%	+59.4%	+50.6%	+29%	+39.9%	+57.9%
ASC-US+ORG VS. ASC-US-ORG p VALUE	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001

Women with ASC+ORG were significantly younger than women with ASC-ORG, but the differences in prevalences of all HPV types, HR-HPV, HPV16/18 and biopsy results of CIN1+ and CIN2+ persisted even when the data were analyzed by age groups.

Conclusions: We found significant increases in HPV prevalence and abnormal biopsy results in women with ASC-US+ORG.

454 Subclassification of "Follicular Lesion of Undetermined Significance" in Thyroid Fine-Needle Aspirates

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Background: Follicular lesion of undetermined significance (FLUS) is a category in the Bethesda system for reporting thyroid cytopathology that encompasses a heterogeneous group of lesions that contain cells exhibiting a degree of architectural and/or nuclear atypia that exceeds expected benign changes but is not of sufficient magnitude to justify classification into other categories. It has been suggested that FLUS could be further subclassified into more distinct subtypes each conferring a different magnitude for the risk of malignancy.

Design: We performed a computerized search of our information system and identified all thyroid fine-needle aspiration (FNA) cases carrying a diagnosis of "atypical follicular cells" that had a follow-up lobectomy or thyroidectomy. The FNA cases were re-reviewed and subclassified into four subgroups: FLUS cannot exclude follicular neoplasm (FLUS-FN), FLUS cannot exclude Hürthle cell neoplasm (FLUS-HCN), FLUS cannot exclude papillary carcinoma (FLUS-PTC) and FLUS, not otherwise specified (FLUS-NOS). Based on the follow-up surgical pathology results, the risks of papillary microcarcinoma (PMC), malignancy (not including papillary microcarcinoma) and neoplasm-NOS (including all malignant tumors, PMC and follicular adenoma) were calculated for each of the 4 FLUS subgroups. The data were analyzed using the t-test.

Results: A total of 138 FLUS cases with surgical pathology follow-up were subclassified into 48 cases of FLUS-NOS, 41 cases of FLUS-PTC, 32 cases of FLUS-FN and 17 cases of FLUS-HCN. The risk of PMC was 22% for FLUS-PTC, 18% for FLUS-HCN, 10% for FLUS-NOS, 9% for FLUS-FN and 15% for all FLUS cases. The risk of malignancy was 32% for FLUS-PTC ($p < 0.05$), 25% for FLUS-FN, 9% for FLUS-NOS, 0% for FLUS-HCN ($p < 0.05$) and 18% for all FLUS cases. The risk of neoplasm-NOS was 81% for FLUS-FN, 68% for FLUS-PTC, 53% for FLUS-HCN, 44% for FLUS-NOS and 60% for all FLUS cases.

Conclusions: In our study, subclassification enabled us to further divide FLUS cases into high-risk and low-risk groups. The high-risk group includes FLUS-PTC and FLUS-FN with malignancy risks of malignancy of 32% and 25% respectively. FLUS-HCN has a low risk profile with a risk of malignancy that was similar to that of the benign thyroid nodule but with an 18% risk of PMC and 53% risk of neoplasm. After excluding all specific FLUS subtypes, the remaining FLUS-NOS group demonstrates only a 9% risk of malignancy which is well within the 5-15% malignancy risk suggested for FLUS as described in the original Bethesda system.

455 Cytohistologic Correlations of 124 Hürthle Cell Lesions

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Background: Hürthle cell lesions are common in thyroid FNA. This study is to find out the frequency of various cytologic features in Hürthle cell lesions and correlated with histology.

Design: We have performed on-site assessments for > 10,000 ultrasound-guided thyroid FNAs since 1995. 124 out of 239 (51.8%) cases of Hürthle cell-rich, lymphocyte absent aspirates had histology for examination and are the basis of this study.

Results:

Clinical Data

Histology	Cases	Age	M:F	Cm
HCa, widely invasive	7	33-91(70)	4:3	4-12.5(6.3)
HCa, angioinvasive	6	31-89(53)	1:5	1.6-5(3)
HCa, capsular invasion only	13	25-77(54)	5:8	1.1-5.2(2.7)
Adenoma	64	23-87(53)	18:46	0.7-7(2.8)
Adenomatoid nodule*	22	25-68(49)	5:17	0.7-4.8(2)
Thyroiditis	12	27-70(49)	0:12	1-4.5(2)

HCa: Hürthle cell carcinoma; *Hürthle cell nodule, unencapulated and no coexisting thyroiditis

Cytologic and Histologic Features

Pathology	Macrofollicles on Histology	Micro-follicles	Isolated-cell pattern	Small cell dysplasia	Large cell dysplasia	Transgressing blood vessels	Thin colloid
HCa, widely invasive	0(0%)	3(43%)	3(43%)	4(57%)	3(43%)	2(29%)	0(0%)
HCa, angioinvasive	3(50%)	3(50%)	1(17%)	1(17%)	1(17%)	0(0%)	1(17%)
HCa, capsular invasion only	4(31%)	7(54%)	4(31%)	6(46%)	4(31%)	5(38%)	1(8%)
Adenoma	21(33%)	32(50%)	9(14%)	22(34%)	10(16%)	15(23%)	10(16%)
Adenomatoid nodule	12(55%)	10(45%)	3(14%)	5(23%)	0(0%)	2(9%)	7(32%)
Thyroiditis	4(33%)	10(83%)	0(0%)	0(0%)	2(17%)	0(0%)	4(33%)

Figure 1 Angioinvasive HCa with abundant colloid in cytology and histology.

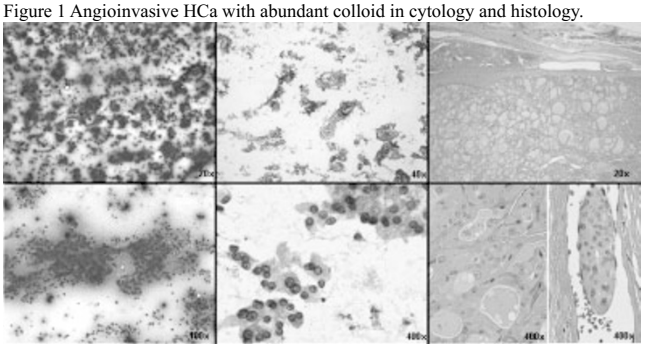
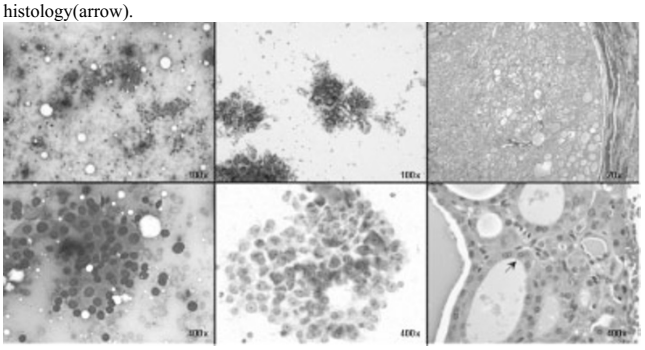


Figure 2 Adenomatoid Hürthle cell nodule with small cell dysplasia in cytology and histology(arrow).



Conclusions: Abundant colloid may rarely occur in HCa. Small cell dysplasia, large cell dysplasia, isolated-cell pattern, microfollicles and transgressing blood vessels may occur in adenomatoid Hürthle cell nodules. Histologic study shows that macrofollicles are frequent in Hürthle cell tumors and the lining cells along the macrofollicles have scanty cytoplasm.

456 Cervical Cytology and High Risk HPV Genotype Distribution in Blacks

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Background: Persistent infection with high-risk (HR) human papilloma-viruses (HPV) has been demonstrated as causal factor for cervical neoplasia. Molecular testing for HR HPV is now recommended as part of initial triage for women 30 years and older. Cervista™ HPV HR (HPV HR) and genotyping 16/18 (HPV 16/18) are FDA-approved HPV tests currently in clinical use. To date, literature on HR HPV distribution in Blacks is still wanting. Here we present our initial findings on the HR HPV genotype distribution among black women in Brooklyn, NY and correlate their cervical cytology results.

Design: Of 138 HPV HR positive patients from 716 total patients (19.3%) referred for HPV HR testing from January to March 2011, only 47 Blacks with sufficient remaining cervical specimens in ThinPrep solution were processed for HR HPV genotypes by HPV 16/18 and Linear Array (LA) HPV Genotyping kit (Roche). Cytology results were correlated with HPV genotype. HPV 16/18 and LA genotyping results were also compared.

Results: The 47 patients ranged from 19 to 75 years old (mean age = 42.6 yo). Twenty-one patients (44.7%) had cytology diagnosis of ASC-US and above, including 16 ASC-US (34.0%), 1 ASC-H (2%), 2 LSIL (4.3%) and 2 HSIL (4.3%). Among patients with abnormal cytology, LA HPV 59 (28.6%) and HPV 16 (23.8%) were more common. Multiple HPV genotypes were present in 19 (40.4 %) patients determined by LA tests and 14 (73.7%) had abnormal cytology. Three of 6 patients with abnormal cytology had single HPV genotype. From combined HPV 16/18 and LA genotyping, HPV 16 (19.1%) is the most frequent genotype, followed by HPV 59 (14.9%). HPV 18, 35, 39, 51 and 82 had similar prevalence, infecting 6% of this population. The infection prevalence pattern was similar in women under 30 yo and above. HPV 16 was positive in 9 patients by HPV 16/18 but only 1 was confirmed by LA. Three of 8 HPV 16 negative patients had current or previous cytology diagnosis of LSIL or HSIL. All three HPV 18 positive were confirmed by LA. The concordance between HPV HR and LA test was only 44.7% (21/47).

Conclusions: From this ongoing study, our initial results showed that abnormal cytology was associated with concurrent multiple HPV genotypes, with HPV 16 and 59 being the most prevalent. Though Brooklyn's black population is 50% Carribeans, HR HPV distribution in our cohort differs from that published on Caribbean or Caucasian US populations. However, it resembles the urban adolescent population in Georgia which is mostly African American. Furthermore, the Cervista™ HPV test is more sensitive compared to LA test in detecting HPV 16.

457 Immunocytochemical Detection of HER2 in Urine Cytology in Previously Papanicolaou-Stained Slides Is Comparable to Correspondent Urothelial Carcinoma Tissue Samples

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Background: Reported rates of HER2 overexpression in urothelial carcinoma of the urinary bladder (UCB) varies widely from 10 to 80%. Majority of studies have been performed on tissue or cell-block samples with little information available on the reproducibility of the test in previously Papanicolaou-stained slides. Detection of HER2 may become routine in UCB patients, since Trastuzumab combined chemotherapy has proven to be a feasible treatment in advanced or metastatic UCB patients in a multicenter Phase II NCI trial. Preferably, HER2 testing is performed on tissue samples, however, if tissue is limited or not available, knowledge of reproducibility of the test in stored cytology samples is desirable. The aim of this study was to compare HER2 expression by immunocytochemistry (ICC) in previously Papanicolaou-stained slides with corresponding tissue samples in patients with UCB.

Design: Thirty-six urine cytology slides and corresponding tissue samples from patient with high-grade UCB were tested for HER2 by ICC and immunohistochemistry, respectively. C-erbB-2 polyclonal antibody (Dako) was tested on alcohol-fixed, previously Papanicolaou-stained slides and in tissue sections, using the L-SAB detection system. In tissue sections, criteria used for a positive result was based on the score system currently available for breast carcinoma. Moderate to strong homogeneous membrane staining (3+) over 30% of tumor cells in the tissue sections was considered positive. In cytology slides, a positive result was recorded when more than 30% of isolated tumor cells showed strong homogeneous membrane staining.

Results: HER2 overexpression was detected in 23 of 36 (64%) urine cytology samples and in 28 of 36 (78%) histology samples. In 20 cases, HER2 was detected in both, urine and correspondent tissue. Of the eight equivocal (2) and negative (6) cases on tissue, 3 were positive in urine slides. Positive predictive value for the detection of HER2 by ICC in urine samples is 87%.

Conclusions: Detection of HER2 over-expression in urine cytology by ICC positively correlates with tissue section results. Previously Papanicolaou-stained slides from urine samples can be used for HER2 ICC detection when tissue sample is scarce or not available.

458 Detection of Chromosomal Abnormalities by Fluorescence In Situ Hybridization on Ultrasound Guided Fine-Needle Aspiration Samples from Pancreas

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Background: Diagnosis of pancreatic malignancy is frequently established on cytological specimens acquired by endoscopic ultrasound guided fine-needle aspiration (EUS-FNA). However, the sensitivity of cytology depends on several factors such as: tumor characteristics, experience of cytopathologists, and endosonographer. Previous studies have demonstrated genetic alterations in pancreatic carcinoma including mutations in oncogenes such as KRAS, chromosomal instability and inactivation of tumor suppressor genes such as p16 on 9p. The aim of this study is to verify the significance of cytogenetic abnormalities by fluorescence In situ hybridization (FISH) in EUS-FNA from patients with pancreatic mass.

Design: EUS-FNA procedures were performed on 81 patients who presented with pancreatic mass between January 2009 and March 2011. Samples were submitted for cytology and cytogenetic studies using the commercially available UroVysion kit. FISH detects aneuploidy for chromosomes 3, 7, 17 and loss of p16 gene at 9p21. Malignancy by FISH is defined by more than 20% cells with 2 or more chromosomes with extra-copies or deletion of 9p21 in more than 20% cells. The final diagnosis was made on clinical follow-up, cytology, and histological samples.

Results: Out of 81 patients, 12 showed benign conditions (chronic pancreatitis, autoimmune pancreatitis), 1 solid and cystic pseudopapillary tumor, 5 neuroendocrine tumors, 7 intraductal papillary mucinous neoplasms, 1 metastatic renal cell carcinoma, 1 mucinous cystadenoma, and 54 adenocarcinomas. In 47 out of 54 (87%) adenocarcinomas, cytogenetic abnormalities were detected by FISH. Loss of p16 was observed in 81% (38/47) of cases as heterozygous deletion (43%, 20/47) or homozygous deletion (38%, 18/47). Loss of P16 was not seen on IPMN-related or endocrine carcinomas. The second most common abnormality was gain of chromosome 3, 7 and 17. FISH detected 13 additional cases of pancreatic adenocarcinoma missed by cytology. All (12) benign conditions were negative by FISH.

Conclusions: Loss of tumor suppressor gene P16 and chromosomal instability are frequent events in the pancreatic ductal adenocarcinoma. FISH analysis on EUS-FNA specimens is a useful and specific diagnostic tool to detect adenocarcinoma.

459 Solid Pseudopapillary Tumor of the Pancreas: Spectrum of Clinical Presentations and Morphologic Variants

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Background: Solid pseudopapillary tumor (SPT) of the pancreas is a rare neoplasm predominantly seen in young women. It typically presents as a large tumor with cystic and solid components. The major differential diagnosis includes pancreatic endocrine

tumor (PEN). This study presents our experience with this tumor with emphasis on two morphologic variants: SPT with signet ring cells, and SPT with clear cells.

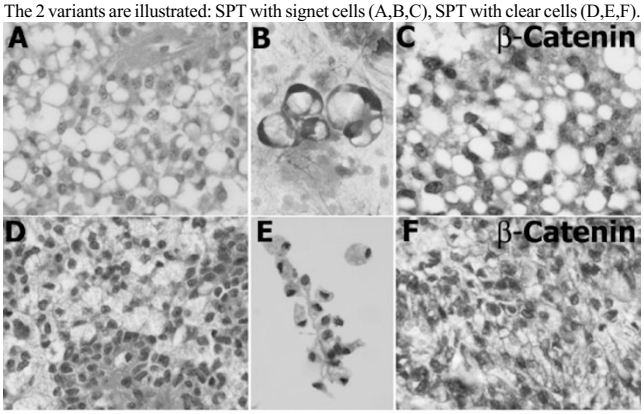
Design: Fifteen histologically confirmed SPT were identified in our files. Endoscopic ultrasound-guided fine needle aspiration (EUS-FNA) was performed in 8/15 cases. Patients' demographics, cytohistologic correlation and tumor characteristics were evaluated.

Results: The results are summarized in the table.

Age (yr)	Sex	Site	Size (cm)	FNA Dx	Gross
23	F	Body/tail	1.7	Nondiagnostic	Solid
36	F	Body/tail	2.3	SPT vs. PEN	Solid
27	M	Body/tail	3.6	Malignant with signet ring features	Solid
36	F	Body/tail	3.5	SPT	Solid
55	F	Body/tail	3.5	SPT vs. PEN	Cystic
50	F	Body/tail	8.5	SPT	Solid & cystic
39	F	Head	1.5	SPT	Solid
25	F	Body/tail	2	SPT with vacuolated cells	Solid & cystic
17	F	Head	3	ND	Solid
45	M	Body/tail	NA	ND	Solid
23	F	Head	6.5	ND	Solid
24	F	Body/tail	8.5	ND	Cystic
42	F	Body/tail	NA	ND	NA
73	M	Body/tail	3.2	ND	Solid
38	M	Body/tail	2.7	ND	Solid

ND - not done; NA - not available

11/15 subjects were female and 4 were male with an age range of 17-73 years. 12 SPT were located in the pancreatic body/tail, and 3 in the head. Tumor size ranged from 1.5-8.5 cm and 10 were solid. Of the 8 EUS-FNA, 4 were diagnosed as SPT, 2 as SPT vs. PEN, 1 as malignant with signet ring features, and 1 was nondiagnostic. Immunohistochemistry (IHC) was performed on cell blocks in 6/8 FNAs. Panels included β-catenin, CD10, vimentin, CD56, synaptophysin, chromogranin and keratin. The 2 variants are illustrated: SPT with signet cells (A,B,C), SPT with clear cells (D,E,F).



A, B, C - SPT with signet ring cell features. D, E, F - SPT with vacuolated cytoplasm.

Conclusions: SPT may occur in males and older adults, and present as a small or solid tumor. Variants with signet cells or clear cytoplasm may pose a diagnostic challenge. However, when combined with the appropriate IHC studies, an accurate diagnosis can be provided by FNA and histologically. Awareness of the wide spectrum of its clinical presentations and morphologic variants can prevent diagnostic pitfalls.

460 Should LSIL-H Be a Distinct Cytology Category? A Study on Frequency and Distribution of 40 HPV Genotypes in a Cohort of Underserved Women

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Background: The Bethesda System (TBS 2001) for gynecologic cervical cytology reporting classifies squamous intraepithelial lesions (SIL) into low grade (LSIL) and high grade (HSIL) lesions. In clinical practice, an intermediate term LSIL-H has been used in a small percentage of LSIL cases with atypical squamous cells insufficient qualitatively or quantitatively to diagnose HSIL. However, the diagnostic criteria of LSIL-H are not defined and little is known about HPV status in those patients. We therefore analyzed the frequency and distribution of 40 HPV genotypes among expanded cytology categories including LSIL-H.

Design: A total of 808 SurePath specimens were collected from women who were referred to our institution from 01/2000-4/2011 for abnormal Pap tests. The patients' average age was 36.5 years (range 19-85 yr). The cytologic interpretations included NILM (n=497), ASCUS (n=48), ASC-H (n=9), AGC (n=2), LSIL (n=165), LSIL-H (n=27), HSIL (n=56), adenocarcinoma (n=1) and unsatisfactory (n=3). HPV DNA was extracted from residual SurePath specimens and amplified with polymerase chain reaction (PCR) in the L1 region. HPV genotypes were determined by DNA microarray against 40 HPV subtypes followed by a confirmatory sequencing assay.

Results: Patients with LSIL-H had much higher frequency of high risk HPV (HR-HPV) infection (92%) than those with NILM (52%), ASCUS (72.9%), ASC-H (77.8%), LSIL (74.3%), or LSIL/ASC-H combined (74.4%). The frequency of HR-HPV infection in LSIL-H was strikingly close to that in HSIL (91.1%). HPV 16, the most common carcinogenic HPV type, was present in a much larger fraction of LSIL-H (36%) than in LSIL/ASC-H combined (13.8%), but in a smaller fraction than in HSIL (44.6%). Furthermore, LSIL-H and HSIL had similar fractions of low and intermediate risk HPV subtypes which were lower than in LSIL or LSIL/ASC-H combined. The HPV distribution patterns did not differ significantly between younger (<30 yr) and older (>=30 yr) age groups.

Conclusions: The patients classified as LSIL-H had a higher risk for HR-HPV infection which was similar to patients with HSIL and much higher than those with ASCUS, ASC-H, LSIL or LSIL/ASC-H combined. The differences were independent of patients' age. Recognizing LSIL-H as an independent diagnostic category may help in early identification of a higher risk subgroup in LSIL who may require a management algorithm comparable to HSIL.

461 Frequency and Distribution of 40 HPV Genotypes in Uninsured Latino Women with Abnormal Pap Tests

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Background: Knowledge about the prevalence and distribution of HPV genotypes in cervical premalignant and malignant lesions is crucial to guide development of clinical management strategies and of prophylactic vaccines. The aim of this study was to determine the frequency and distribution of HPV genotypes in an underserved cohort of women.

Design: From 1/2010 to 4/2011, 808 SurePath cervical specimens were collected from uninsured Latino women who were referred to our institution for abnormal Pap tests. The patients' average age was 36.5 years (range 19-85 years). The specimens were tested for 40 HPV genotypes by DNA microarray and sequencing assay.

Results: In this underserved cohort of women, the HPV infection rate was high with frequent multi-strain infection (38.4%). The combined frequency of HPV16/18 was 55.1% in HSIL. HPV6 and 11 were infrequent (2.9% and 1.1%). The frequency of HPV 90 was unexpectedly high (2.4%) and associated with dysplasia (9.4%).

Conclusions: The frequency and distribution pattern of HPV genotypes in this cohort differs from previously published US data. HPV 90, an uncommon genotype in the US was identified in the cohort. Understanding the differences and possible changes in HPV distribution pattern may help guide the development of appropriate preventative and therapeutic strategies targeting underserved population.

Table 1. Frequencies and Distribution of 40 HPV Genotypes in Major Cytology Categories

Cytology Diagnosis	No HPV (%)	LR-HPV% (S/M)*	IR-HPV% (S/M)	HR-HPV% (S/M)	Total HPV infection %	Most frequent HR-HPV types (in decreasing order)
NILM (n=497)	9.5	28 (37/23)	13 (15/12)	58 (48/64)	90.5	16, 18, 53, 52, 39, 45, 66, 67, 90, 56
ASCUS (n=48)	8.3	28 (32/27)	8 (0/9)	64 (68/62)	91.7	16, 18, 45, 51, 52, 53, 42, 35, 59, 67
ASC-H (n=9)	11	0 (0/0)	33 (20/43)	67 (80/57)	89	31, 53, 18, 39, 52, 58, 66
LSIL (n=167)	1.2	24 (23/24)	18 (17/18)	59 (60/58)	98.8	53, 56, 16, 18, 66, 58, 39, 51, 67, 82
LSIL-H (n=25)	0	15 (0/24)	6 (11/3)	78 (89/72)	100	16, 58, 51, 45, 39, 31, 53, 67, 18, 33
HSIL (n=56)	1.8	18 (10/22)	5 (0/7)	77 (90/69)	98.2	16, 31, 18, 45, 39,56, 58, 59, 53, 43
Total‡	7.1	26 (31/24)	13 (13/13)	62 (56/63)	92.9	16, 18, 53, 56, 39, 58, 45, 52, 66, 67

*Percentage of HPV infection (Single strain infection/Multi-stain infection); LR-HPV, IR-HPV and HR-HPV represent low, intermediate and high risk HPV, respectively. ‡ include rare AGC (2) and cancer (1) cases (not shown).

Dermatopathology

462 microRNAs as Prognostic Biomarkers in Malignant Melanoma

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Background: MicroRNAs (miRs) are important regulatory molecules. Many recent advances have shown their dysregulation in cancer. Our laboratory has previously reported altered expression of key miRs in melanoma. To develop a further understanding of the clinical importance of such miRs, we have assembled a cohort of primary melanoma patients.

Design: The Agilent miRNA microarray platform was used to generate global miRNA expression profiles for 66 primary melanoma tissue samples. The tumours were from single institution cases, with uniform treatment and follow-up protocols for which clinical data were available. Using supervised analyses, we looked for association of expression level for each miR with pathological (Breslow depth less than or equal to 2mm versus greater than 2mm and low mitotic count versus high mitotic count) and clinical (no metastatic progression versus presence of distant or regional metastasis and alive versus deceased) endpoints.

Results: We identified numerous miRs whose expression level appeared associated with both clinical and pathological endpoints, in most cases showing lowered expression in the more aggressive disease. Of particular interest, we found that expression of miR-150 as well as members of the miR-200 family were significantly associated with the pathological endpoints measured. These miRs were relatively downregulated in thicker melanomas and those with high mitotic rates compared to the thinner or less mitotically active tumours. When clinical endpoints were assessed, lower expression of miR-150 was also strikingly correlated with death and with the presence of metastatic disease.

Conclusions: We conclude that miR expression levels measured in the primary diagnostic lesion can be used to prognosticate clinical outcome in melanoma. The miR-200 family and miR-150 appear to be most promising in this regard. We are currently investigating a role for these miRs and their putative target mRNAs in melanoma progression and outcome, using experimental, statistical and bioinformatics approaches.