Design: A search of the autopsy records from 1993 to 2008 for coronary dissections including both native and bypass grafts was performed.
Results: A total of 17 cases of dissections of the coronary arteries and bypass grafts were found. There were 10 women and 7 men with age ranging from 28 to 82 (median age of 62). Dissections of native coronary arteries (11 cases; 8 female:3 male) involved the left coronary arteries in all but 1 patient. Four patients had secondary coronary dissection occurring as an extension of acute ascending aortic dissection while iatrogenic etiology was due to PCI (4) and aortic valve replacement (2). One patient with segmental mediolytic arteriopathy who died of hemoperitoneum was found to have dissection of the left circumflex artery. There were 6 cases (2 female:4 male) of dissections of bypass grafts identified with involvement of the internal mammary artery graft in 4 and saphenous vein graft in 2. Five patients in this latter group underwent coronary artery bypass grafting and one occurred as a complication of aortic valve replacement. Majority of patients (59%) died of myocardial infarction within 1 to 4 days.
Conclusions: Iatrogenic and secondary dissections of native coronary arteries were more common in females and usually involves the left coronary arteries. In contrast, dissections of bypass grafts was observed more frequently in males and were associated with cardiac surgery. Segmental mediolytic arteriopathy is a rare cause of spontaneous coronary dissection.

372 Right Atrial Tumors and Tumor-Like Lesions, a Clinicopathologic Study of 54 Cases

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Background: Cardiac tumors are rare, and those arising in the right atrium form a heterogenous group of tumor and tumor-like conditions that have not yet been studied extensively in a single series.
Design: Fifty-four cases of tumors arising in the right atrium were collected retrospectively from 1995 to 2009 from the files of the Armed Forces Institute of Pathology. The clinicopathological characteristic of benign and malignant proliferations of tumors primarily centered in the right atrium were studied.
Results: Patients range from 6 to 81 years (mean 48.8, SD 18), with 28 males and 26 females. Tumor size ranged from 3 to 20cm. Most common presentation was shortness of breath (18%), followed by chest pain (11%), and other included hemoptysis, pericardial effusion, syncope and arrhythmias. Neurologic symptoms were present only in myxomas (2 cases). Ten cases were incidentally found on imaging or during surgery. There were 31 benign proliferation that included 9 thrombus, 5 fibroinflammatory proliferations (2 of which were associated with clinical and histologic features of sclerosing mediastinitis), 4 cardiac myxomas, 4 lipomatous hypertrophy of the atrial septum, 2 hamartomas of mature myocytes, 2 paragangliomas, 1 nodular tuberculous infiltrate, 1 inflammatory myofibroblastic tumor, 1 cardiac fibroma, 1 hemangioma and 1 leiomyoma. Malignant tumors were 16 primary sarcomas (13 angiosarcomas, 2 synovial sarcomas, 1 pleomorphic sarcoma), 4 lymphomas and 3 metastases (2 melanomas, 1 metastatic adenocarcinoma). Clinical presentation, age, tumor size and sex did not correlate with malignant diagnosis.
Conclusions: Right atrial tumors represent a variety of benign and malignant condition with varied clinical presentation and histologic appearance. Most are benign and include non-neoplastic proliferations. Malignant cases are more homogeneous with angiosarcomas most common, followed by lymphoma and metastases.

373 Immunohistochemical Expression of N-Cadherin, but Not Plakoglobin and Plakophilin, Is Decreased in Pathologically Diagnosed Autopsy-Based Cases of Arrhythmogenic Right Ventricular Cardiomyopathy

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Background: Arrhythmogenic right ventricular cardiomyopathy (ARVC) is a genetic disorder related to mutations in desmosomal proteins. There have been few histologic studies quantitating expression of desmosomal proteins in autopsy samples of AC.
Design: We studied 29 patients (20 males, 9 females) dying suddenly with ARVC. Their age at death was 33 ± 4 and 32 ± 6 years, respectively. Control subject tissues were 15 men and 7 women, mean ages 36 ± 4 and 32 ± 6 years, respectively. Sections of heart were taken from short axis cuts: 25 sections from control tissues (10 right ventricle-RV, 12 left ventricular free wall-LV, and 3 ventricular septum-VS) and 58 sections from AC (26 RV, 23 LV, and 9 VS). Areas free of fibrofatty change were assessed. Immunohistochemical stains against plakoglobin, plakophilin, and N-cadherin were applied and area expression analyzed by both computerized morphometry correlated with a three-point scale of staining intensity and semi-quantitatevely independently by two pathologists.
Results: The mean area of plakoglobin (5.7%) and plakophilin (5.4%) showed no difference by gender, cardiac site (left ventricle, right ventricle and ventricular septum), patient age, or presence of ARVC. The mean area of N-cadherin staining showed no difference by gender (5.1 ± 0.5% in men vs. 5.1 ± 0.3% in women, p=.99), age (p=.08), or cardiac site (p = 0.7). The mean % staining of N-cadhrein by morphometry was 6.7 ± 0.4% in controls (range 2-14%, vs. 4.5 ± 0.3% in ARVC (ragne 1-14%), p=.0009. A positive predictive value of weak (1+ staining) for ARVC was 76%, with a negative predictive value of 50% (88% sensitivity, and 30% specificity, respectively). By multivariate analysis, % N-cadherin staining was inversely proportional with the presence of ARVC, independent of other variables (p=.0001).
Conclusions: We conclude that immunohistochemical evaluation of plakophilin and plakoglobin does not distinguish ARVC from controls. The relationship between specific mutations in cases of pathologically proven AC and the expression of desmosomal-related proteins needs to be further evaluated. N-cadherin is significantly decreased, but as a qualitative measure is not a specific diagnostic tool.

374 Osseous Metaplasia in Calcific Aortic Valve Stenosis: Role of Congenital Bicuspid Valves

PA VanderLaan, RF Padera, FJ Schoen, RM Mitchell. Brigham and Women's Hospital, Boston, MA.
Background: Calcific aortic valve stenosis (CAVS) is prevalent in aging populations, and is the most common pathology in aortic valves (AVs) requiring surgical excision; the pathogenesis is uncertain and controversial. We correlated the clinical and pathologic characteristics of AVs excised for CAVS that also demonstrate osseous metaplasia (OM).
Design: A retrospective analysis was performed for 2087 native AVs excised for CAVS at Brigham and Women's Hospital from January 2003 to July 2009. Cases of active endocarditis were excluded, as were fragmented valves where cuspal number could not be evaluated; 37 glutaraldehyde-pretreated bioprosthetic AVs excised for calcific degeneration were also examined, implanted from 2-24 years (mean 11.3). Patient demographics were correlated with histologic features (including OM) as diagnosed on paraffin embedded, formalin-fixed, decalcified H&E stained sections.
Results: Among 2087 native AVs with CAVS, 1597 (76%) were tricuspid (TC), 486 (23%) were bicuspid (BC), and 4 (0.2%) were unicuspid, with an overall M:F ratio of roughly 1.5:1. Overall, OM was present in 15% of valves with CAVS. OM was approximately twice as common in BC valves versus TC valves (23.5% vs. 12.6%, Z=5.85), with an earlier mean age of excision (59.7 vs. 75.6 years, p<0.0001). OM was also more common in valves removed from males versus females, in both BC (26.6% vs. 21.7%, Z=2.29), and TC valves (14.3% vs. 10.5%, Z=2.31), with an earlier age of excision (BC: 58.9 vs. 61.6 years, p<0.01; TC: 75.0 vs. 76.3 years, p<0.01). Notably, OM was not observed in any bioprosthetic AVs excised for calcific degeneration.
Conclusions: OM is a common finding in native AVs excised for CAVS, and is more frequent in congenital bicuspid valves. The higher frequency of OM in BC valves with CAVS at an earlier age suggests an inherent susceptibility of these congenitally abnormal valves, possibly attributable to mechanical effects or an intrinsic molecular phenotype of the valve cells. The higher incidence of OM in men further suggests a role for sex hormone modulation of the metaplastic process. The absence of OM in non-viable bioprosthetic AVs suggests osteoblastic transformation of intrinsic cells rather than recruitment of circulating stem cells. These findings can direct future mechanistic studies in unraveling the pathogenesis of CAVS.

375 Ascending Giant Cell Aortitis without Systemic Symptoms Is Associated with an Increased Frequency of Subsequent Distal Aortic Events

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Background: In patients undergoing resection of the ascending aorta for aneurysm or dissection, giant cell aortitis (GCA) is occasionally identified upon pathologic examination. Frequently, ascending GCA is present without systemic systems of vasculitis (GCA-WSS). The clinical significance of GCA-WSS has been unclear. Specifically, it has not been established if patients with ascending GCA-WSS are at increased risk for more distal aortic events on long-term follow-up.
Design: Ascending aortic segments resected for aneurysm or dissection between 1980 and 2004 were reviewed. GCA-WSS was identified by the presence of granulomatous inflammation with or without giant cells. Exclusion criteria included prior or concurrent evidence of systemic rheumatologic disease, age less than 50, mycotic / infectious aortitis, lymphoplasmacytic aortitis, and follow-up of less than fours years duration. Twelve GCA-WSS cases with adequate follow-up were identified. For each GCA-WSS case, 2 non-aortitis control ascending aorta cases were identified. Aortitis cases and controls were matched for age, gender, indication for surgery, date of surgery, type of surgery, and follow-up duration. In both groups, follow-up included imaging with CT and/or MR for the majority of the patients. Aortic events were defined as operations, new aneurysms greater than 5 cm, ruptured aneurysms, or dissections involving the descending thoracic or abdominal aorta.
Results: 5 of the 12 GCA-WSS patients (42%) experienced distal aortic events during follow-up. There were new large thoracoabdominal or descending thoracic aortic aneurysms in three patients, and ruptured thoracoabdominal aneurysms in two additional patients. Three of these 5 patients underwent subsequent aortic surgery. In contrast, only 1 out of 24 patients (4%) in the non-aortitis group had an event, a new thoracoabdominal aneurysm. The difference in the frequency of events is statistically significant, P=0.01.
Conclusions: In long-term follow-up, patients with GCA-WSS are at significantly increased risk for distal aortic events compared with non-aortitis patients. In GCA-WSS patients, subsequent aortic events occur most often in the descending thoracic aorta and/or suprarenal abdominal aorta rather than the infrarenal abdominal aorta.

Cytopathology

376 Cytologic Features of Thyroid Lesions Diagnosed as Indeterminate for Neoplasia Which Predict Follicular Neoplasm on Fine Needle Aspiration Biopsy of Thyroid (FNAB): A 5-Year Retrospective Study in a Tertiary Care Hospital in Ontario, Canada

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Background: Follicular lesions (FL) of the thyroid encompass non-neoplastic and neoplastic lesions. Diagnosis of follicular lesion is a challenging area in the interpretation

of thyroid FNAB. Objectives: The purpose of the study is two-folds: 1). To identify cytologic features that will more accurately predict neoplasia (FN) and 2). To recognize diagnostic pitfalls in the differential diagnosis of follicular lesions (FL).
Design: A computer database search was performed for FL, indeterminate for neoplasia on FNAB of thyroid between January 2003 and August 2008 from the archives of the Cytopathology section at our institution. A total of 504 cases were retrieved with a diagnosis of FL; 205 (40.6%) had a histological follow-up and were retained for the study.
Results: Histological follow-up of 205 cases showed follicular Adenoma (FA) in 53 (25.8%), hurthle cell adenoma (HA) in 19 (9.2%), multi-nodular goitre (MGN) in 95 (46.3%), papillary carcinoma, follicular variant (PTCFV) in 25 (12.2%), thyroiditis (THY) in 8 (3.9%) and follicular carcinoma (FC) in 5 (2.4%) patients. Cytologic features that accurately predict FA (48 specimens, 91%), FC (4 specimens, 80%) and PTCFV (23 specimens, 92%) are tight microfollicle formations (defined as acinar structures formed by crowed enlarged nuclei with chromatin clearing and inconspicuous nucleoli) present in >60% of the smears and scant to nil colloid. Another important cytological fearture than we found in our study is the presence of nuclear grooves in more than 5% of follicular cells, which predicts presence of neoplastic lesion. However, presence of nuclear grooves in more than 30% of follicular cells are predictive of papillary carcinoma. Nuclear grooves in more than 5% of follicular cells were found in FA (16 specimens, 30%), FC (2 specimens, 40%) and PTCV (16 speicmens,64%) on FNAB. The most common diagnostic pitfall is follicular cells wrapped up in clotted blood and endothelial cells (MGN, 87 specimens).
Conclusions: The cytologic features that will increase diagnostic specificity of FN (FA, FC and PTCFV) are tight microfollicle formations present in more than 60% of the smears, nuclear grooves in >5% of cells and scant colloid. The most common diagnostic pitfall is clotted blood.

377 Cytology as Primary Diagnostic Modality in the Diagnosis and Classification of Neuroendocrine Neoplasms in Various Body Sites: A Nine Year Retrospective Review

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Background:: A nine year retrospective study was conducted by collecting and analyzing all the neuroendocrine carcinomas (NEC) diagnosed by cytology from various body sites.
Design: The tumors falling into the realm of neuroendocrine (NE) differentiation, whether by morphology or immunohistochemistry were identified by using cytology data base. These included small cell carcinoma (SCC), carcinoid, large cell neuroendocrine carcinoma (LCNEC), neuroblastoma, paraganglioma, pancreatic endocrine neoplasm (PEN), medullary carcinoma of thyroid (MCT), and NEC not otherwise specified (NOS). These cases were followed up by review of medical records.
Results: 148 cases of NEC diagnosed by cytology were identified. These included 110 cases of SCC, 12 cases of carcinoid, four cases of LCNEC, three paragangliomas, five MCT, five neuroblastomas, one PEN and eight NEC, NOS. The common cytological mode of diagnosis were image or bronchoscope guided fine needle aspiration biopsy (FNAB), core biopsy with imprint cytology, FNAB of peripheral palpable lesions and occasionally effusion cytology. In 72 cases (48.6%) additional material related to the primary diagnosis in the form of concurrent surgical biopsy or cytological specimen was available. SCC was by far the most common diagnosis (74.3%), with 65 cases being primary in the lung and 45 cases were metastatic,especially liver. Concurrent biopsy or cytological material was obtained in 52 cases (47.2%) of SCC and in 40 cases (36.3%) it supported the diagnosis. Two of three cases called suspicious for SCC on cytology had corresponding endobronchial biopsies which were positive. Discrepancy was seen in five cases (3.3%). Two cases called LCNEC by cytology had biopsy showing Non-small cell carcinoma, and SCC. Another case diagnosed as SCC, was an olfactory neuroblastoma. Two cases suspicious for MCT were confirmed as hyperplastic goiter by surgical resection. Cytohistologic correlation was almost 100% in carcinoid, paraganglioma and neuroblastoma.
Conclusions: This study emphasizes the utility of cytology as a primary mode of diagnosis of NEC. Cytological approach in the form of FNAB is the most useful technique for a rapid, accurate, less invasive and cost effective approach. Recognizing the NE pattern of tumor and then effectively triaging the specimen for ancillary studies enables rapid diagnosis and prompt treatment.

378 Diagnostic Utility of CK5/6 and P63 in Fine Needle Aspiration of the Breast Lesions Using Cell Block

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Background: Core biopsy has recently replaced fine needle aspiration cytology (FNAC) in the assessment of breast lesions. The challenge in interpretation of breast FNAC with frequent ambiguous diagnoses and false positive and false negative cases are among factors leading to decline in the use of breast FNA. Previous studies have shown that basal-type cytokeratin (CK5/6) can distinguish benign from malignant epithelial proliferations in histology. In this study, we explore the diagnostic value of cytokeratin CK5/6 and p63 in a series of breast FNAC.
Design: Breast FNA cases with corresponding cell block diagnosed as proliferative breast lesion (PBL) with atypia and PBL without atypia were retrieved for a 5 year period. All cases had a subsequent lumpectomy with pathologic diagnosis. A cytologic diagnosis of PBL with atypia or PBL without atypia was used if the cytomorphologic findings did not fit a more specific category. We also included 10 cases of fibroepithelial lesions for comparison. Immunostaining for CK5/6 and p63 were performed on cell block of all cases. The percentage of immunoreactive cells was graded as follows: 0 (0-5%), 1+ (6-25%), 2+ (26-50%) and 3+ (>50%).
Results: Sixty four breast FNA cases were included in the study. Cases were interpreted as PBL with atypia in 29/64 (45%) and PBL without atypia in 35/64(55%). In PBL with atypia, 9/29 (31%) were found to be carcinoma on resection, and 7/35 (20%) cases in PBL without atypia. In FNAC (cell block) specimens, CK5/6 was negative (score 0) in 8/11 (73%) and score 1+ in 3/11 (27%) cases of histologically-proven invasive carcinoma. In situ carcinoma, 3/5 (60%) cases were score 0 and 2/5 (40%) scored 1+. In benign breast lesions, CK5/6 scored 3+ in 40/48 (83%), confirming its benign nature. CK5/6 was negative in 8/10 (80%) cases of fibroepithelial lesions. P63 stained 58/64 (91%) cases without significant difference between malignant and benign cases. However, p63 showed characteristic staining of bipolar naked nuclei in the background of the specimen from fibroepithelial lesions (not appreciated with CK5/6).
Conclusions: CK5/6 is of diagnostic value in differentiating between carcinomatous and non-carcinomatous cytologic atypia in breast FNA. Although CK5/6 may be of limited value in fibroepithelial lesions, p63 staining could be helpful in the diagnosis of these cases. Thus, CK5/6 and p63 can help increase the diagnostic accuracy of FNA of breast lesions.

379 The Potential of Telecytology of Cell Block Preparations from Pap Smear Samples "TelePAPology"

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Background: Background Digital images are being used for telecytology, automated screening of Pap smears, training and education, as well as, proficiency testing. However, to date, the impact of the digital imaging on routine day to day cytology remains far from perfect. Cell block (CB) preparations from the discarded/residual conventional and liquid based GYN samples have been shown to be feasible, valid and of diagnostic value. Furthermore CBs could be used for detection of HPV DNA by both immunohistochmistry and in-situ hybridization, thus eliminating the need of separate testing. The aim of this study is to evaluate the feasibility of CB using imaging technology to overcome current limitations with digitizing cytologic specimens. Our goal is to present this novel approach as a model for widespread adoption to achieve accurate diagnosis, provide high quality preparations, consistent results and timely reports.
Design: H&E stained CB specimens prepared from residual samples of Pap smears were used. Cellient automated CB system from Hologic (Marlborough, MA) was used to prepare CBs. 31 cases consisting of 11 atypical squamous cells of undermined significance (ASCUS), 12 low grade squamous intraepithelial lesion (LGSIL) and 8 high grade squamous intraepithelial lesion (HGSIL) have been evaluated thus far. Slides were scanned using the Aperio digital imaging system (Vista, CA). They were reviewed manually and digitally by pathologists and cytotechnologists. Correlations were performed between digital and manual CB reads and routineThin prep reads for sensitivity and specificity.
Results: CBs contained optimal amount of material from all cases for meaningful evaluation. There was 100% concordance between manual and digital reads of CBs for both pathologists and cytotechnologists. Compared to thin prep reads, 9 of 11 ASCUS cases were reclassified as LGSIL using CBs and 2 cases of LGSIL were upgraded to HGSIL. Time required for digitally scanning CB slides varied from 2-3 minutes. Average time spent reviewing slides was similar to conventional method (2-5 min).
Conclusions: Digital evaluation of CB prepared from pap samples is a feasible method for widespread adoption to achieve high quality specimen preparations, consistent, reliable and timely diagnosis that can potentially reduce the biopsy load significantly especially in a resource-poor settings. It has the potential to revolutionize the screening for cervical cancer.

380 Is 'Bronchoalveolar Carcinoma' a Diagnosis That May Be Made on FNAC?

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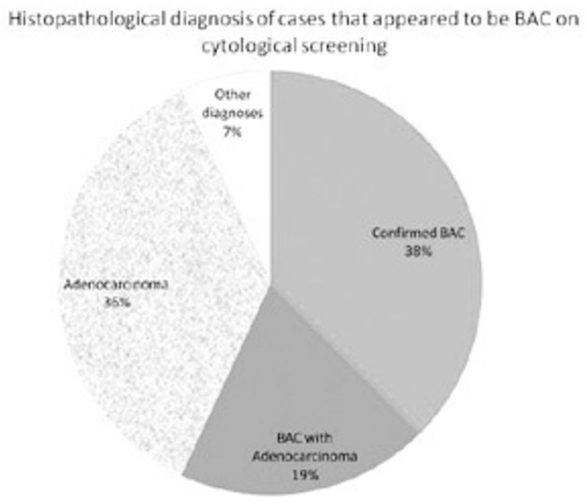
Background: Bronchoalveolar carcinoma (BAC) is considered an in situ lesion where surgical resection is curative. It presents as a space occupying lesion in the lungs with ground glass opacity and is often biopsied for cytological evaluation. Demonstration of a well-differentiated, localized tumor with lepidic growth pattern suggests a BAC. But well differentiated adenocarcinoma or an adenocarcinoma of mixed subtype with invasive patterns can also give a similar deceiving picture. It is not known whether cytologic diagnosis of BAC indeed correlates with future histological diagnosis.
Design: We did a retrospective search of all cases that were diagnosed as 'BAC' following FNAC at the Ottawa Hospital ('00 -'09). All cases needed to have a subsequent wedge resection or lobectomy with evaluations done at our institution. Correlations between cytological and subsequent histopathological diagnosis was then evaluated.
Results: 72 cases that also had a subsequent surgical resection were selected. Among these, only 27 (38%) were later confirmed as BAC. 26 cases (36%) were found to be invasive adenocarcinomas, 14 cases (19%) had a mixed subtype of both BAC and adenocarcinoma, and the remaining (5%) had multiple other malignant diagnoses.
Conclusions: The study shows that cytologic diagnosis of BAC is inaccurate in >60% of cases. Misdiagnosis of an invasive adenocarcinoma as BAC may give a wrong sense of relief to the clinician and patient, and even delay treamtment. Since BAC is mostly a diagnosis of exclusion that cannot be made without histologic evaluation, we propose that, 'BAC' diagnosis should not be made on cytology alone, but rather needs a combined clinical, radiological and histo-pathological evaluation. Further studies are required to identify reasons for the discrepancy between radiological, cytological and histopathological diagnosis of BAC.



381 Identification of an Effective Antibody Panel in the Diagnosis of Pancreatic Ductal Adenocarcinoma on Fine Needle Aspiration Biopsy Specimens

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Background: Fine needle aspiration biopsy (FNAB) of the pancreas is often the diagnostic procedure of choice to establish the diagnosis of pancreatic ductal adenocarcinoma (PDA). However, the interpretation of FNAB specimens can be challenging due to overlapping cytological features between reactive conditions and PDA. Our unpublished data demonstrated that maspin, KOC, S100P and pVHL were the best antibody panel selected from 26 antibodies reported in the literature and tested to differentiate PDA from benign/reactive pancreatic ducts on surgical specimens. In this study, we further explore the utility of these 4 markers in confirming a benign or a malignant diagnosis in FNAB specimens on cell block preparation.

Design: Immunohistochemical evaluation of maspin, S100P, KOC, and pVHL was performed in 67 cases of FNAB specimens of the pancreas on cell block sections. The 67 cases were divided into 3 groups: Group 1 - 44 cases of PDA; Group 2 - 13 cases with a suspicious or indeterminate diagnosis; and Group 3 - 10 benign cases. The staining intensity (weak or strong) and distribution (negative, 1+, 2+, 3+ or 4+) were recorded. Follow-up surgical or clinical data confirmed the malignant diagnosis for all cases in group 1. Surgical or clinical follow-up data were available in 9 cases in the suspicious group, and all 9 cases were malignant.

Results: A strong and diffuse staining (3+ or 4+) for maspin, S100P, and KOC in 33 (79%), 35 (80%) and 19 (48%) cases in Group 1 and 9 (70%), 7 (54%), and 5 (39%) cases in Group 2 was seen. The 3 pVHL-negative cases contained very low cellularity. Maspin and S100P positive cases in the benign group were gastric contaminants. The results are summarized in Table 1.

Table 1. Summary of Immunohistochemical Staining Results

Groups	Maspin +	S100P +	KOC +	pVHL +
PDA	42/42* (100%)	44/44 (100%)	37/40* (93%)	0/42* (0%)
Suspicious	13/13 (100%)	13/13 (100%)	10/13 (77%)	0/13 (0%)
Benign	1/10 (10%)	2/10 (20%)	1/10 (10%)	7/10 (70%)

*2 cases in the maspin and pVHL groups and 4 in the KOC group did not contain tumor cells in the deeper sections.

Conclusions: Our data indicate that maspin, S100P, KOC and pVHL are an effective antibody panel to confirm a benign diagnosis or PDA on FNAB specimens, which in turn will reduce the number of cases in a suspicious/indeterminate category. Caution should be taken because gastric contaminants can be positive for maspin and S100P.

382 Assessment of EGFR Mutation Status in Needle Biopsies and Cytology Specimens of Lung Adenocarcinoma by Immunohistochemistry Using Antibodies to the Two Major Forms of Mutant EGFR

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Background: Mutations of epidermal growth factor receptor (EGFR) gene in lung adenocarcinoma (AD) predict sensitivity to EGFR tyrosine kinase inhibitors (TKI). Small deletions in exon 19 and the L858R point mutation in exon 21 account for 85-90% of EGFR mutations. Molecular testing is best performed on surgically resected tumors but many patients are not operative candidates and are diagnosed by cytology or needle biopsy only. Such tiny specimens are often insufficient for molecular testing in these patients who need systemic chemotherapy or EGFR TKIs. Recently, immunohistochemistry (IHC) with mutation-specific monoclonal antibodies against the L858R mutant and the exon 19 mutant (common 15bp deletion; E746_A750del) has been shown to be sensitive and specific for EGFR mutation status. Here, we evaluated these antibodies in the setting where they would be uniquely useful, cytology or needle biopsy specimens.

Design: We identified 94 lung AD patients with known EGFR status (based on molecular testing of resected tumor) and positive needle biopsy (BX) or cytology (ThinPrep (TP) or cell block(CB)) samples. These 94 samples included 47 with ex19 deletion [deletion sizes: 15bp (n=32), 18bp (n=9), 12bp (n=1), 24bp (n=1)], 35 with L858R, and 12 EGFR wild type cases. IHC with the two EGFR mutation-specific antibodies (Cell Signaling Technology) described above was scored as negative (0-1+) or positive (2+-3+). Both antibodies were performed on EGFR wild type cases. Twelve known negative cases were used as controls.

Results: The sensitivity for EGFR ex19 (15bp only), EGFR ex 19 (including all deletions) and EGFR exon 21 mutation-specific antibodies are 79%, 57% and 66%, respectively. Both antibodies showed a specificity and PPV of 100%. No wild type cases showed immunoreactivity with either antibody.

IHC for EGFR ex19 del and L858R mutant

		IHC for ex19 del	
Mutation Status	Specimen	POS(%)	NEG(%)
ex19 (15bp del)	BX n=22	18(92)	4(8)
	TP n=5	5(100)	0
	CB n=7	4(57)	3(43)
ex19 (all bp del)	BX n=9	0	9(100)
	CB n=4	0	4(100)
		IHC for L858R	
L858R	BX n=12	10(83)	2(17)
	TP n=4	3(75)	1(25)
	CB n=19	10(53)	9(47)

Conclusions: IHC with EGFR mutation-specific antibodies on small biopsies and cytology specimens shows high specificity and good sensitivity for EGFR mutation status. Application of these antibodies could allow or expedite critical treatment decisions in patients with unresectable lung AD.

383 Diagnostic Accuracy of Fine-Needle Aspiration Biopsy of the Parapharyngeal Space

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Background: Fine-needle aspiration biopsy (FNA) of the parapharyngeal space (PPS) is a diagnostic challenge. Sampling is difficult (often trans-oral FNA), primary PPS tumors are rare, and there is a broad differential diagnosis, including salivary gland, neurogenic, carotid body, lymphoreticular, and soft tissue tumors. PPS FNA has rarely been studied, with only four series of more than 20 cases, all from major referral centers, reporting high rates of diagnostic accuracy (87-100% accuracy, sample size 24 to 63 FNAs). It is unclear whether this high rate of diagnostic accuracy can be achieved in a smaller tertiary care centre with fewer CT-guided FNAs and a lower volume of routine head and neck cytology.

Design: Pathology records from our institution (an 1100 bed Canadian academic tertiary care centre) were searched to identify all patients who underwent PPS FNA from Sept. 1991 to Aug. 2009. Records were reviewed for all cases. The FNA diagnosis was compared to the gold standard of subsequent histopathology or long term clinical follow up.

Results: 27 patients (12 male, 15 female, mean age 53 years) had 36 FNAs (9 patients had repeat FNAs). 33 FNAs were office based and 3 were done under image guidance. 11/36 (31%) FNAs were non-diagnostic. In the 25 diagnostic FNAs, there was sensitivity 89%, specificity 94%, PPV 89%, NPV 94%, and accuracy 92% for the diagnosis of positive or negative for malignancy. A correct specific diagnosis (e.g. "schwannoma") was made in 9/25 (36%) cases. The non-diagnostic rate was significantly higher ($p<0.025$) in FNAs prepared as conventional smear cytology (9/17 = 53%) versus liquid based ThinPrep cytology (2/19 = 11%). A specific diagnosis was made significantly more often ($p<0.05$) with ThinPrep (8/19 = 43%) versus conventional (1/17 = 5.9%). Comments about specimen quality were less frequent with ThinPrep (2/19 = 11%) than with conventional (8/17 = 47%), where obscuring blood and degeneration were frequently cited by pathologists. One minor complication from FNA occurred, temporary facial nerve palsy secondary to xylocaine.

Conclusions: Our institution has a high rate of diagnostic accuracy for classifying PPS FNAs as benign or malignant, but a lower rate of reporting a specific diagnosis. Non-diagnostic FNAs are frequent and occur more often with conventionally prepared smears than with ThinPrep. Improved specimen quality with ThinPrep seems to be a factor, but other confounding variables not studied here may contribute to this difference.

384 Anal and Pharyngeal Screening Cytology and Human Papilloma Virus Testing in HIV-Infected Men Who Have Sex with Men

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Background: The incidence of anal squamous dysplasia and cancer in men HIV-infected who have sex with men is increasing. Anal cytology and HPV testing is proposed as a tool for diagnosing and selecting patients for further anoscopy. Moreover, limited data is available on oral and anal associated diseases in HIV positive patients. The presence of HPV in pharyngeal and anal samples remains to be determined, despite oral cancer associated HPV is known.

Design: Our aim is to correlate results of anal and pharyngeal screening cytology with HPV testing and subtyping in HIV-infected men who have sex with men to establish the significance in anal and oral HPV associated lesions.We collected 94 cases of anal and pharyngeal liquid cytology (Thin-Prep) from 47 HIV-infected men.Cytology samples were classified according to the Bethesda criteria. HPV DNA detection was performed by PCR amplification (HPV Clinical Arrays Genomica). Subtypes were classified according to low (LR), high (HR), potentially high and indetermined risk category.

Results: Cytology: 69 (73'4%) with negative results; 25 (26'6%) abnormal smears: 23 anal samples- 11 (AIS/ASCUS),11 LSIL and 1 HSIL; 2 pharyngeal samples presented AIS/ASCUS.HPV DNA was detected in 61 cases (64'9%);42 anal and 19 pharyngeal (22 with single subtype and 39 multiple infection.The most common were HR-HPV:16(13 cases), 59(12 cases), 35(8 cases) and 52(7 cases). Potential HR 1, indeterminate-risk 3 and LR 4 cases.Multiple infection (39 cases) detected HR subtypes in all samples. HPV was present in 18 cases in both anal and pharyngeal samples. Cytology/HPV Concordance between abnormal cytology and HPV was present in 24 cases (22 anal

and 2 pharyngeal). In pharyngeal positive cases cytology and HPV was present with same HPV in anal sample (52,56).HPV59 was the most frequent subtype in pharyngeal cases. In anal samples predominant multiple infections (36 cases) of HR-HPV were present and 16 was the most prevalent.

Conclusions: Anal predominant multiple subtype infections presented a HR-HPV, therefore it is suggested a more accurate cytologic follow-up and high-resolution anoscopy for this patients.HPV59 is prevalent in pharyngeal samples, although nasopharynx cancer is related to HPV16. Pharyngeal cytology has even lower sensitivity than anal.HPV detection adjuncts to anal and pharyngeal cytology can help to select high risk patients.

385 High Risk HPV Detection and Follow-Up Findings in Women with Vaginal ASC-US Pap Tests

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Background: Unlike the cervical smears, the role of High risk (hr)HPV testing in the triage of vaginal smears with ASCUS is not well known. The significance of hrHPV testing in women with vaginal ASC-US Pap test has very limited discussion in the literature. Most health organizations do not recommend cytologic screening of the vaginal cuff for most women with hysterectomy. In this study we analyzed vaginal ASC-US cases and correlate hrHPV status with histologic and Pap follow up (F/UP) findings.

Design: From our computer database, for a study period of 49 mths from July 2005 to July 2009, all the vaginal smears diagnosed with ASCUS and tested for hrHPV were retrieved. All Thin Prep Pap Test (TPPT) were prepared by an automated processor and imaged using Thin-prep imager. hrHPV detection was performed by Hybrid Capture 2 (HC2) method. F/UP results of biopsies and Pap tests were recorded. The most significant abnormality was recorded.

Results: A total of 1125 cases of vaginal TPPT were interpreted as ASCUS and also had hrHPV testing results. Of those, 244 cases (21.7%) were hrHPV positive, and 881 cases (78.3%) were hrHPV negative.138 hrHPV positive women had histologic and/or Pap F/UP results. Among hrHPV negative group, 379 cases were randomly selected to check the F/UP results, and 235 women had histologic and/or Pap F/UP results. The results are listed in the table 1. The study subjects were of an average age of 57yrs (20-91). F/UP period ranged from 0.2-44 months with a median of 22 months. Overall, VAIN 2/3/ HSIL (5.1%) and VAIN1/LSIL (42.8%) detection rates were significantly higher in hrHPV positive women than in hrHPV negative women (P<0.001). There was no statistically significant difference in VAIN detection between women ≤54 years and ≥55 years.

Follow-up Results

	Total			hrHPV Positive		hrHPV Negative	
F/UP	F/UP NO.	LSIL-VAIN1*	HSIL/-VAIN2/3*	LSIL-VAIN1*/ Total cases	HSIL-VAIN2/3*/ Total cases	LSIL-VAIN1*/ Total Cases	HSIL-VAIN2/3*/Total Cases
Cytology	302	27 (8.9%)	0	22/84 (26.2%)	0	5/218 (2.3%)	0
Histology*	71	42 (59.2%)	8 (11.3%)	37/54 (68.5%)	7/54 (12.9%)	5/17 (29.4%)	1/17 (5.9%)
Total	373	69 (18.5%)	8 (2.1%)	59/138 (42.8%)	7/138 (5.1%)	10/235 (4.3%)	1/235 (0.4%)

Conclusions: 1. There is close correlation of hrHPV status and VAIN lesion detection in women with vaginal ASC-US Pap tests, with the similar pattern as is seen in the cervical ASC-US Pap tests. 2. Reflex hrHPV testing is strongly recommended for women with vaginal ASC-US Pap tests. 3. Colposcopy should be performed for women with hrHPV positive vaginal ASC-US Pap tests.[table1

386 The Utility of Cytologic Specimens for Molecular Testing, Our Experience in a Cancer Center

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Background: With increased targeted treatment for cancer patients, PCR-based molecular testing to determine the eligibility for the treatment has been significantly increased in our hospital. However, the experience of using cytologic specimens for molecular testing is limited. We retrospectively reviewed the cytology cases with molecular testing in order to determine the utility of cytologic specimens for the testing.

Design: A total of 130 molecular tests using cytologic specimens from 62 patients were retrieved from the Department of Pathology at M.D. Anderson Cancer Center from June to August in 2009. The patients included 26 males and 36 females with an age-range from 37 to 87 years old and an average age of 63 years. The cytologic preparations, i.e., cell blocks vs. smears, were compared with the specimen adequacy and DNA extraction sufficiency for molecular testing.

Results: In 62 patients, lung cancer (31 cases) was the most frequent primary carcinoma, followed by colon (5 cases), breast (3 cases), pancreas (2 cases) and ovary carcinoma (1 case). Other malignancies include melanoma (8 cases) and lymphoma (5 cases). EGFR was the most frequently tested biomarker (41) followed by K-ras (40), BRAF (14), PIK3CA (8), C-Kit (8) and N-ras (6). Specimens consisted of 56 cases of FNA, 5 cases of pleural fluid and 1 case of peritoneal fluid. In cytologic specimens used for molecular testing, cell block material accounted for 76% (47/62), while smears accounted for 24% (15/62). Insufficient material for molecular testing was determined in 9 cases, 6 cases (13%, 6/47) from cell block material and 3 cases (20%, 3/15) from smears. Six cases had insufficient DNA for molecular testing, 3 cases (6%, 3/47) from cell block material and 3 cases (20%, 3/15) from smears. The combined insufficiency rates for molecular testing were 19% (9/47) in cell block material and 40% (6/15) in smears, respectively.

Conclusions: Cytologic material can be used for molecular testing to guide targeted treatment in cancer patients. Cell block material was used more frequently and had lower insufficient rate for molecular testing compared with smears. Our findings suggested the importance of obtaining sufficient cytology sample for cell block preparation if molecular testing is anticipated.

387 Phosphoprotein Quantitation Via Quantum Dot Immunofluorescence and Multispectral imaging Using Liquid-Based Cytology System: Adapting a Clinical Laboratory Platform for Biomarker Studies

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Background: Recently we have assessed feasibility of quantitative analysis of phosphoproteins in paraffin embedded fixed tissues. In this study we evaluated conditions required in a liquid based cytology (LBC) system that will preserve phosphoproteins (PPs) for IF quantitation.

Design: Lymphoma cell lines treated with hydrogen peroxide to modulate PP levels of pERK1/2, pS6, and pSTAT3 served as a model system. An FDA-approved LBC system (ThinPrep™) with CytoLyt™ (wash/transfer buffer) and PreservCyt™ (preservative/fixative) served as the test clinical laboratory model platform. Imaging was performed using a multispectral imaging system (Nuance™). In order develop QD IF absolute quantitation, we applied an ELISA assay to determine absolute concentrations of pERK.

Results: First, we evaluated the effect of exposure of cells to PreservCyt™ on phospho ERK level and demonstrated good correlation (R= 0.98, P= 0.01) compared to Western Blot quantitation. Next, we tested the effects of pre-treatment of cells with CytoLyt™ (15 min) prior to exposure to PreservCyt™. This resulted in a marked loss (71% on average) of QD IF signal in all analyzed phosphoproteins. Time course studies for exposure to PreservCyt™ indicate that overnight (18 hrs) exposure is optimal for QD IF staining with 80% decrease of pERK signal after 48 hours compared to 18 hrs incubation. To optimize signal we subsequently studied the effect of adding 2% paraformaldehyde to PreservCyt™ and found an approximately 1.4 fold increase in pERK signal at 18 hrs. Interestingly we found optimal QD IF signal with best morphologic preservation by sequencing 10% buffered formalin fixation (30 min) followed by 18 hrs incubation in PreservCyt™ and storage of prepared slides in 96% EtOH. As a proof or principle for an absolute quantitation assay, we performed pERK Elisa and QD IF on paired samples and demonstrate that values of QD IF correlate with ELISA-based measurements. (R= 0.98, P= 0.02).

Conclusions: These preliminary results indicate feasibility of adapting a clinical LBC platform for cytology based in situ quantitative phosphoprotein assays. These studies have implications for 1) monitoring of targeted therapies (such as novel kinase inhibitors) using a minimally invasive FNA technique during early phase clinical trials and 2) predictive theranostic assays by ex vivo demonstration of active pathways that are the target of specific therapies. Further proofs of concept studies are ongoing.

388 Diagnostic Value of DOG-1 Expression in Fine-Needle Aspiration Cytology of Gastrointestinal Stromal Tumors (GISTs)

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Background: DOG-1 is a newly introduced immunohistochemical (IHC) marker that is useful in the diagnostic evaluation of GISTs. DOG-1 gene transcripts were originally identified as a typical finding in gene expression profiling studies on GISTs. The corresponding protein has been recently identified as a calcium-regulated chloride channel protein, encoded by a locus at chromosome 11q13. The utility of DOG-1 as an immunohistochemical marker has very recently been established in the surgical pathologic evaluation of suspected GISTs. It has been shown that DOG-1 immunohistochemistry should be added into the diagnostic panel evaluating surgical pathology specimens from suspected GISTs, especially those GISTs that are C-Kit (CD117) negative. However, there is a lack of data regarding the diagnostic usefulness of DOG-1 in fine-needle aspiration (FNA) cytology of suspected GISTs.

Design: A total 22 cases of GISTs with FNA cytology smears and corresponding cell blocks were retrieved from the hospital computer system. All cases were confirmed by histology with immunohistochemical studies. DOG-1, CD117, and CD34 IHC was performed on cell blocks that were formalin-fixed, paraffin embedded and stained using a heat-induced epitope retrieval technique.

Results: 19/22 patients (86.4%) had GISTs that were CD117 positive and DOG-1 positive. Of those 19 patients, 4/19 (21.1%) were CD34 negative. 3/22 patients (13.6%) had GISTs that were DOG-1 positive and CD117 negative. Of those 3 patients, all were positive for CD34. DOG-1, CD117, and CD34 all showed membranous and cytoplasmic staining. Of the cases that were DOG-1 and CD117 positive, DOG-1 showed stronger staining versus CD117.

Conclusions: Our results indicated that DOG-1 is a sensitive and specific marker in diagnosing GISTs in FNA cytology specimens, especially in C-kit negative cases. We believe that an IHC panel consisting of CD117, DOG-1 and CD34 is very useful to confirm a diagnosis of GIST in FNA cytology and can confirm the diagnosis of GIST in C-kit negative cases.

389 Comparison between the Bethesda and the British Thyroid Association-Royal College of Physicians Systems for Reporting Thyroid Cytopathology: Experience in Two Hospitals

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Background: Thyroid FNA has proven to be one of the most effective tools for guiding the initial management of patients with thyroid nodules. The new 6-tiered Bethesda System for Thyroid FNAs sponsored by the National Cancer Institute and the 5-tiered British Thyroid Association - Royal College of Physicians thyroid FNA System offer two approaches to the problem of reporting thyroid FNA results. The efficiency of these 2 systems has particular significance for the European cytology community. In this study, we present the combined experience from our institutions for reporting thyroid FNAs using these two different systems and evaluate their efficacy based upon surgical follow up.

Design: Data on thyroid FNAs and their corresponding surgical specimens were collected at our two institutions over a two-year period. We compared the sensitivity

and specificity for each of the component groups within the 2 systems where a diagnosis lead to surgery (CAT 3, 4, 5, 6 and THY 3, 4, 5 versus benign).

Results:

Table 1

	Bethesda system	British system
Patients	210	289
Nodules	294	386
Age (range)	51 (7-88)	50 (16-86)
Sex (F/M)	178/32	234/55

Table 2

Bethesda system	FNA (%)	Positive histology	Negative histology	British system	FNA (%)	Positive histology	Negative histology
CAT		20 (28%)	51 (72%)	THY		26 (37%)	44 (63%)
1	31 (10.5)	0	4	1	34 (8.8)	2	4
2	156 (53)	2	15	2	306 (79.3)	2	24
3	4 (1.4)	/	/				
4	79 (27)	5	28	3	24 (6.2)	3	14
5	13 (4.4)	4	4	4	6 (1.6)	5	1
6	11 (3.7)	9	0	5	16 (4.1)	14	1§

§1 benign nodule in Hashimoto thyroiditis

The sensitivity for CAT 3, 4, 5, 6 and for THY 3, 4, 5 were almost equal in the two reporting systems, Bethesda and British, (90% vs 91.6%), while specificity was higher in the British (31.9% vs 60%). Interestingly, the number of cases in the CAT 4 and THY 3 categories (suspicious/indeterminate) differed significantly between the two reporting systems (27% and 6.2%). For these two categories, the rate of malignancy on surgical excision is similar (18% and 21%), with a PPV of 15% and 17% respectively.

Conclusions: The two reporting systems, the Bethesda and the British, show similar sensitivities but the British system exhibited better specificity, possibly related to differences in the use of the so called "grey zone" (CAT 4 and THY 3) categories. Larger studies will be useful to further confirm these data.

390 Withholding Reflex HPV Status: Diagnostic and Financial Implications

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Background: No previous studies have directly examined how the information made readily available to the pathologist at the time of sign-out impacts the ASC-US and reflex HPV (rHPV) positivity rates of an institution. Specifically, does withholding the knowledge of whether the patient has a rHPV test (rHPVt) ordered have an impact on the diagnosis, and therefore should institutions set up the flow of their case sign-outs to include or exclude this information? Our department invariably provides on the requisition sheet of PAP smears whether the patient has a rHPVt ordered, which accounts for 21% of all of the HPV testing performed at our institution.

Design: To determine whether the ordering status of a rHPVt should be made available at the time of case sign-out, this knowledge was withheld from the pathologist during sign-out for a study period of six weeks. The ASC-US and positive rHPV rates were then compared from the study period to the rates obtained during the control period, defined as the same six-week interval the prior year.

Results: During the study period, 2713 PAP smears were reviewed, of which 215 (7.9%) were signed out as ASC-US, with an ASC-US to LSIL ratio of 1.3. Of these, 79 had rHPVt were ordered, of which 35 (44.3 %) were positive. During the control period, 3362 PAP smears were reviewed, with 301 (9.1%) diagnosed as ASC-US, and an ASC-US to LSIL ratio of 1.26. Of these, there were 105 rHPVt, of which 32 (30.5%) were positive. Thus, withholding the rHPVt ordering status resulted in a higher HPV positive predictive value for ASC-US diagnoses.

Conclusions: The lower rHPV positivity rate during the control period may be attributed to overcalled diagnoses, in order to give the patient the benefit of a rHPVt, thus diluting the ASC-US category with cases which are negative. Given the direct cost to the patient for HPV testing at our institution is $70.00, this practice leads to financial waste for the healthcare system. Comparing our study period to the control period's positive rHPVt rates, and assuming similar patient demographics, the financial waste approximates $8,805.77 annually for our institution. As healthcare costs and its impact on patient care is at the forefront of both political and healthcare system discussions, it is beneficial to set-up practices in such a way that will not only decrease costs, but also improve patient care. Thus, it would behoove departments to restrict knowledge regarding the rHPVt ordering status from cytopathologists during sign-out, in order to prevent unnecessary influence on the final diagnosis.

391 Fine Needle Aspiration Cytology of Intraductal Papillary Mucinous Neoplasm of the Pancreas: A Study of 128 FNA Cases with Correlating Histopathology

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Background: Intraductal papillary mucinous neoplasm (IPMN) is considered to be a precursor lesion for some pancreatic adenocarcinomas. The preoperative cytologic evaluation of patients with suspected IPMN helps assist the clinician in therapeutic decision-making. In this study, we assess the accuracy of the FNA diagnosis of IPMN and refine our criteria for the cytologic diagnosis of this entity.

Design: A computerized search of our laboratory information system was performed for the 14-year period ending June 30, 2009 to identify all cytology and surgical pathology cases in which IPMN was diagnosed or considered in the differential diagnosis. All reports and all available FNA and surgical pathology slides were then retrospectively reviewed.

Results: Of 128 patients in whom both FNA and pancreatic resection were performed, a final histologic diagnosis of IPMN was rendered in 119 cases. For 40 of the 119 histologically confirmed IPMN cases (34%), the preoperative FNA diagnosis was IPMN. For the remaining 79 histologically confirmed IPMN cases (66%), the FNA diagnoses included: extracellular mucin (25 cases, 21%), benign lesions (22 cases, 18%), cyst/pseudocyst (3 cases, 2.5%), negative/contaminant/unsatisfactory (11 cases, 9%), atypical cells (4 cases, 3%), mucinous cystic neoplasm [MCN] (8 cases, 7%), IPMN or MCN (1 case, 1%), adenocarcinoma arising in an IPMN (1 case, 1%), mucinous adenocarcinoma (3 cases, 2.5%), and adenocarcinoma NOS (1 case, 1%). In 9 of the 128 cases, an FNA diagnosis of IPMN was rendered, but not confirmed by histology. The histologic diagnoses for those 9 cases included: benign lesions (3 cases, 33%), mucinous ductal ectasia (1 case, 11%), mucinous neoplasm (2 cases, 22%), adenocarcinoma (1 case, 11%), adenocarcinoma with PanIN (1 case, 11%), and PanIN (1 case, 11%).

Conclusions: In our series, the sensitivity of FNA for the diagnosis of IPMN was relatively low with only 40% of histologically proven cases having received an accurate preoperative FNA diagnosis of IPMN. Some of the IPMNs (7%) were misclassified as MCN by FNA, an error that may be preventable by more careful correlation with imaging findings. Of note, abundant extracellular mucin was detected in an additional 20% of cases, suggesting that the presence of extracellular mucin alone could constitute a cytologic diagnostic criterion for IPMN, when observed in conjunction with appropriate imaging findings.

392 Cytological and Histopathological Associations of Cervical Intrapithelial Neoplasia Grade 2 and Characteristics of Cyclin D1 Expression in Women with Irregular Menstrual Cycle

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Background: Identification of cervical intraepithelial neoplasias (CIN) in routine Pap smears is achieved based on the cytological criteria by the Bethesda System for Reporting Cervical Cytology. However, these criteria do not take into account a phase or regularity of menstrual cycle. We set out to test the hypotheses that irregular period is of importance in diagnosing CIN2, and cyclin D1 is a useful biomarker of cervical dysplasia.

Design: We studied 85 women with cytological diagnosis of CIN2 in cervical Pap smears, including 23 reproductive patients with regular period (group 1), 42 reproductive patients with irregular period (group 2), and 20 postmenopausal patients (group 3). Cervical biopsies were taken from all patients and routine histopathologic examination and immunohistochemistry were carried out using anti-Ki-67 and cyclin D1 antibodies.

Results: Histological examination confirmed CIN2 in 78.3% (18/23) of patients from group 1, 66.7% (28/42) of patients from group 2, and 80% (16/20) of patients from group 3. Immature squamous metaplasia was seen in two patients (8.9%) from group 1, ten patients (23.8%) from group 2 and three patients (15%) from group 3. Basal cell hyperplasia was seen in two patients (8.9%) from group 1, three patients (7.1%) from group two, and one patient (5%) from group 3. CIN3 was found in one patient (4.3%) from group 1 and one patient (2.4%) from group 2. High expression of cyclin D1 was observed in 65.2% (15/23), and low expression in 21.7% (5/23) of women from group 1. High expression of cyclin D1 was seen in 47.6% (20/42), and low expression in 42.9% (18/42) of women from group 2. High expression of cyclin D1 was observed in 40% (8/20), and low expression in 35% (7/20) of women from group 3. Ki-67 was expressed in 2/3 of epithelium in CIN2 and full thickness of epithelium in CIN3.

Conclusions: This study suggests that the discrepancy between cytological and histological diagnoses of CIN2 is primarily observed in women with irregular menstrual cycle, and this clinical information may be taken into account in diagnosing high-grade cervical dysplasia. In these patients cyclin D1 expression was not associated with CIN2, and this biomarker does not seem to provide additional information in differential diagnosis.

393 Molecular Analysis of Pancreatic Cyst Fluid: Correlation with Cytologic Diagnosis and Surgical Follow-Up

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Background: Endoscopic ultrasound-guided fine needle aspiration (EUS-FNA) is increasingly utilized as a preferential tool for the work-up of pancreatic cystic lesion. Clinical management of these cystic lesions is largely dependent on revelation of the nature of cystic lesions. It is however quite challenging to render a diagnosis based on cytomorphologic analysis alone. Few previous studies have demonstrated that ancillary studies such as fluid amylase and CEA levels as well as molecular approaches play an important role in classifying and diagnosing pancreatic cystic lesions. In this study, we retrospectively review our experience with molecular analysis of pancreatic fluid and its correlation with cytologic diagnosis and surgical follow-up.

Design: A total of 82 pancreatic cyst EUS-FNA specimens from 71 patients that had concurrent molecular analysis were retrieved from the cytopathology archives at our institution. RedPath Integrated Pathology performed molecular analysis as a commercially available test. Molecular analysis, based on DNA quality/quantity, K-ras mutation and allelic imbalance mutation, classified cystic lesions into benign serous neoplasm, low, intermediate, and high grade mucinous cystic neoplasm (MCN). Surgical follow-up was available in 20 patients (28%).

Results: Of 82 cases, 20, 4, 37, 21 cases were classified as MCN, adenocarcinoma, bland epithelial cells and cyst content by cytomorphologic analysis. By molecular analysis, 23 cases of cytologically MCN and adenocarcinoma were classified as low, intermediate, and high grade MCN. Eighteen and 11 cases cytologically diagnosed diagnosed as bland epithelial cells were classified as MCN and serous neoplasm, respectively. For cyst content only cases, 13 cases were classified as either MCN or serous neoplasm. Classification render by molecular analysis had a 95% correlation rate with surgical follow up in classification.

Conclusions: Molecular analysis of pancreatic cyst fluid played a significant role in the diagnosis and classification of pancreatic cystic lesions in addition to cytmorphologic analysis.

394 The Role of Cytopathology in the Diagnosis and Management of Pancreatic Cysts Greater Than 3 cm

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Background: The role of cytology in pre-operative evaluation of pancreatic cysts is controversial. Recent guidelines recommend cysts greater than 3 cm be resected without cytologic diagnosis. In this study we evaluate the usefulness of cytology in the diagnosis and management of pancreatic cysts greater than 3 cm.

Design: The study cohort consists of resected pancreatic cysts greater than 3 cm between 2003-2007. Clinical, radiologic and pathologic information was evaluated and a multimodal approach was used to pre-operatively triage cysts for resection. Cysts not needing resection required either 1) benign lesional epithelium or 2) cyst contents without an epithelial component AND no atypical features on EUS (no thick cyst wall, invasion, mural nodule or dilated main pancreatic duct) AND CEA either greater than 192 ng/ml (consistent with a neoplastic mucinous cyst) OR an elevated amylase and low CEA (consistent with a pseudocyst or serous cyst). A cyst needing resection required at least atypical cells consistent with a mucinous cyst with moderate dysplasia or malignant epithelium. Cases were considered non-diagnostic if the fluid contained no epithelial cells, no radiologic information on the cyst was available or CEA was not analyzed. Cyto-histological and statistical analysis was performed.

Results: There were 105 resections of pancreatic cysts larger than 3 cm, 69 females (average age 53.4 years) and 36 males (average age 66.5 years). Thirty-one cysts (29.5%) had prior FNA, 21 females (average age 55.0 years) and 10 males (average age 67.4 years). When compared to histology, excluding six non-diagnostic cases, there were 9 true negatives, 10 true positives, 6 false negatives and 0 false positives. The sensitivity, specificity, positive predictive value and negative predictive value were 62.5%, 100%, 100%, and 60.0% respectively. Seventy-four (70.5%) cysts did not have prior FNA, 48 females (average age 52.6) and 26 males (average age 66.1). Of these cases, 38 (51.4%) were unnecessarily resected and 36 (48.6%) had moderate dysplasia or malignancy.

Conclusions: Patient management based on pancreatic cyst size results in unnecessary surgery in half of patients with pancreatic cysts > 3cm. The combination of cytology, radiology and cyst fluid analysis appropriately triages patients for surgery with high specificity and a PPV of 100%.

395 Dual-Stain for P16 and Ki-67 in the Interpretation of Abnormal PAP Cytology Results: A Prospective Study

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Background: p16 has been found to be strongly over-expressed in nearly all high-grade pre-cancerous and cancerous cervical lesions and may serve as a surrogate marker for the transforming activity of high-risk HPV. As over-expression of the cell-cycle regulatory protein p16 in cells with intact cell cycle regulation should prevent those cells from proliferating, we further tested the hypothesis that the detection of individual cells simultaneously co-expressing p16 protein and proliferation marker Ki-67 can be used as an indicator for the presence of cervical dysplasia. To accomplish this goal we initiated a large prospective study on cervical cytology specimens showing Pap abnormalities.

Design: Residual materials from liquid-based cytology specimens of women attending cervical cancer screening at a major tertiary hospital and reference center over one year period were used for the analysis. Specimens with any abnormal Pap cytology result (ASC-US+) were included. For each case, an additional ThinPrep™ slide was prepared and immuno-stained using a prototypic dual staining reagent kit (**CINtec®Cytology, Dual stain**) for the simultaneous detection of p16 and Ki-67 expression on the same slide. The presence of one or more individual cells co-expressing both p16 and Ki-67 were interpreted as "positive" test result. Follow-up biopsy and HPV results were obtained.

Results: Sensitivity of the Dual stain was 87.0% for CIN2+ and 95.7% for CIN3+, with specificity of 89.8% for non high-grade CIN. In ASC-US/ASC-H/LSIL categories with positive HPV results, a positive Dual stain result identified all CIN3+ cases at high levels of specificity (up to 80%).

Conclusions: Initial results from a first set of cytology specimens subjected to simultaneous p16/Ki-67 dual staining and with biopsy follow-up (n=661) indicate both high sensitivity and specificity of this novel screening approach to detection of CIN2/3+ on biopsy follow-up. Results showing high specificity rates for the Dual stain support this approach as an enhancement for detecting histopathological CIN2/3+.

396 Effectiveness of Rapid Prescreening in Liquid Based Pap Tests

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Background: Rapid prescreening (RPS) has been shown to be an effective quality control procedure for detecting squamous intraepithelial lesions (SIL) in conventional Papanicolaou smears. In the United States RPS is rarely adopted, with experts citing CLIAA'88 regulations that limit cytotechnologists' daily slide volumes. Technologies, including liquid based preparations (LBP) and imaging systems, hypothetically increase SIL detection, potentially lowering the effectiveness of RPS. This study measures the effectiveness of RPS in SIL detection in a U.S. laboratory that uses imaged and non-imaged LBP.

Design: A designated cytotechnologist performed RPS in 3916 LBP Pap tests: 2127 SurePath™ (SP) preparations that were manually screened and 1789 ThinPrep Pap tests™ (TP) that were imaged using the ThinPrep Imaging System™ (TIS). The cytotechnologist manually reviewed each slide for 30 to 120 seconds categorizing the Pap test as Unsatisfactory, Within Normal Limits or Abnormal (ASCUS and above). RPS results were then compared with the results obtained by routine screening. Discordant cases were reviewed by a QC cytotechnologist or pathologist. A 10% negative rescreen was performed in compliance with laboratory and CLIAA'88.

Results: RPS had a 0.5% combined detection of atypical cases for SP and TP specimens. RPS of SP had a sensitivity of 0.45 and a specificity of 0.99 for cases of ASCUS and above; TP had a sensitivity of 0.48 and a specificity of 0.97. For LSIL and above, the sensitivities of SP and TP were 0.72. The sensitivities for HSIL and above for SP and TP specimens were 0.67 and 0.73, respectively. SP and TP had a false negative rate (FNR) of 0.55 and 0.52, respectively. A comparison of negative RPS diagnoses to routine screening revealed a 2% overcall of ASCUS based upon HPV results and pathologist diagnosis. Overall 10% negative rescreen resulted in 6% significant diagnostic discrepancies.

Conclusions: RPS did not result in identifying a significant number of false negative cases. The findings may indicate that this laboratory is composed of a highly sensitive group of screeners, or that there is a tendency to overcall otherwise negative cases. The increased SIL detection of LBP may also limit the effectiveness of RPS. The 2% overcall of ASCUS cases, identified as negative on RPS, may support its use as a quality improvement tool to lower ASCUS/HPV rates. The 10% negative rescreen, especially in imaged cases, was more effective than RPS in SIL detection. The FNRs calculated for the manually screened SP specimens and TIS specimens were not significantly different.

397 Utility of Epithelial-Lined Fibrovascular Cores in Cell Blocks, Squamoid Change, and Hemosiderin for Distinguishing Papillomas from Other Lesions in FNA of the Breast

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Background: Papillomas of the breast are notoriously hard to diagnose by FNA, and there is a need for new criteria. Three previously untested criteria were evaluated for their ability to distinguish intraductal papillomas (IDP) from other lesions: hemosiderin in macrophages, a distinctive squamous metaplasia, and the presence of epithelial-lined fibrovascular cores (FVC) in cell block sections.

Design: We searched our Medical Center's database for breast FNA diagnoses with the term "papillary" or "papilloma" between 2005 and 2009. Of 122 cases we excluded nine cases categorically called positive (all confirmed on follow up) and 65 cases with no follow up. The FNA of the remaining 48 cases were blindly scored for hemosiderin in macrophages, squamoid change (defined as a cell with abundant waxy cytoplasm and perinuclear vacuoles, frequently in worrisome three dimensional groups with increased chromatin), and FVC in cell block sections.

Results: Surgical follow-up showed 28/48 (58%) cases of IDP and 20 cases that were not IDP. Hemosiderin was present in 18/28 (64%) of IDP and 10/20 (50%) cases that were not IDP ($p<.49$). Squamoid change was present in 18/28 (64%) of IDP compared to 8/20 (40%) of cases that were not IDP ($p<.22$). The presence of both squamoid change and hemosiderin was seen in 15/ 28 (54%) of IDP, but only 6/20 cases (30%) that were not IDP ($p<.18$). Cell blocks (all Cellient™) were able to be made from residual material in 22/28 (79%) of IDP cases compared to 12/20 (60%) of cases that were not IDP. On review of available sections, FVC were identified in cell block sections in 16/20 (80%) of IDP cases but only in 2/12 (17%) of cases that were not IDP on follow-up ($p<.0018$). Of the two cases with FVC in cell blocks without IDP at follow-up, both had benign core biopsies; sampling error appears to account for the absence of an IDP. Of the 28 IDP cases, 6 cases (21%) had an associated carcinoma. Diagnoses for the 20 cases that were not IDP on follow-up showed 1 ductal carcinoma.

Conclusions: The presences of hemosiderin and squamoid change are associated with benign papillomas, but are not reliable for diagnosis. FVC's in cell blocks (Cellient™) is the best discriminator of true papillomas, sometimes allowing a definitive diagnosis.

398 Evaluation of Intraoperative Cytology Preparations (ICP) To Differentiate Central Neurocytoma (CN) from Oligodendroglioma (OL) and Ependymoma (EL)

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Background: ICP are used as rapid complements to frozen section examination during intraoperative evaluation of brain tumors. CN is an uncommon discrete intraventricular round cell neoplasm, typically located in the foramen of Monro. In spite of CN's peculiar intraventricular location, the cellular monotony, and vascular network make its distinction from OL and EP, tumors that can involve periventricular areas, a diagnostic dilemma during intraoperative consultations. This distinction is underscored by different critical extent of surgical resections according to the gross-total resectability of the tumor type. The aim of this study is to identify which ICP features can help to distinguish CN from its two mimickers: OL and EP.

Design: All intraoperative cytology preparations of proven cases of CN, OL, and EP between January, 2001 and March 2009 were retrieved. Four cases of CN, six OL, and four EP, all WHO grade II, were independently examined by two cytopathologists blinded to the diagnoses. All diagnoses were confirmed by tumor tissue sections examination by neuropathologists. All CN cases were further supported by synaptophysin or neuron-specific enolase, and glial fibrillary acidic protein immunostains. The following parameters were evaluated: neuropil, nuclear roundness, nuclear monotony, cellular cohesiveness, vascular network, chromatin density, nucleoli, perivascular and fibrillary rossetes, type of cytoplasmic processes, nucleus to cytoplasm ratios, entrapped glia or neurons, and microcalcifications. Statistical discriminant analysis was performed to analyze the findings.

Results: Statistical analysis revealed 100% congruence (0% misclassification) on the presence of either neuropil or diffuse nuclear monotony (less than 10% tumor cell

anisocytosis) to distinguish CN from OL and EP in ICP. Similarly, the presence of short straight capillaries combined with absence of neuropil and diffuse nuclear monotony identified OL; while the lack of short straight capillaries, neuropil, and diffuse nuclear monotony indicated EP.

Conclusions: The presence of neuropil and diffuse tumor nuclear monotony in ICP can be used to distinguish CN from OL, and EP. Alternatively, the absence of these two parameters and the presence of short straight capillaries indicates OL, while the absence of these three parameters identifies EP. ICP can be a valuable aid in differentiating among CN, OL, and EP.

399 Immunohistochemical Expression Profile of Metastatic Urothelial Carcinoma Using Cell Block Cytology: Comparison of Novel Markers S100P and GATA3 to Traditional Urothelial Markers

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Background: Immunohistochemical stains currently used as markers for urothelial differentiation are of limited sensitivity and specificity. S100P and GATA3 are novel markers of urothelial carcinoma identified using DNA microarrays and tissue microarrays (*Am J Surg Pathol 2007; 31: 673-680).* To date, no study using cytologic material has compared these two potential antibodies with traditional urothelial markers namely CK903, p63, uroplakin and thrombomodulin.

Design: 23 patients with clinically metastatic urothelial carcinoma who had positive cytologic specimens with adequate cell block were identified from the files of two institutions. Clinical information was gathered from the electronic medical record. Immunohistochemistry was performed on cell block material using monoclonal antibodies for p63 (Biocare Medical), CK903 (Cell Marque), uroplakin (Fitzgerald), thrombomodulin (Dako), S100P (BD Biosciences) and GATA3 (Santa Cruz Biotechnology. Double immunostaining technique was used for CK903 and p63. The staining intensity was evaluated on a scale of 0 to 3+, and the extent as 1+ to 3+ (<25%, 25-50%, >50%).

Results: There were 18 males and 5 females with mean age of 73 years. Specimens consisted of 15 fine needle aspiration biopsies (lymph nodes 6, liver 3, lung 3, bone 3) and 8 body fluid samples (pleural fluid 5, peritoneal 3). All 23 cases (100%) showed immunoreactivity for thrombomodulin with at least moderate to strong staining in 22 cases (96.5%). CK903 and p63 showed at least moderate intensity of staining in 19 (83%) and 18 (78%) of cases. Uroplakin stained only 1 case (4%). S100P and GATA3 showed cytoplasmic staining in 19 (83%) and nuclear staining in 14 (61%) of cases, respectively. However, staining was more commonly focal with both new antibodies compared with the 3 conventional markers; which tended to show more intense and diffuse staining.

Conclusions: In this study, thrombomodulin is the most useful marker in confirming the urothelial nature of metastatic carcinoma using cell block cytology. CK903 and S100p have similar sensitivity which is superior to either p63 or GATA3. A panel consisting of thrombomodulin and CK 903 with or without p63 or S100p is useful in the work-up of patients suspected of having metastatic urothelial carcinoma using cell block cytology.

400 HPV Genotype Distribution in a Low-Risk Screening Population: Can the Impact of HPV Vaccination Be Demonstrated?

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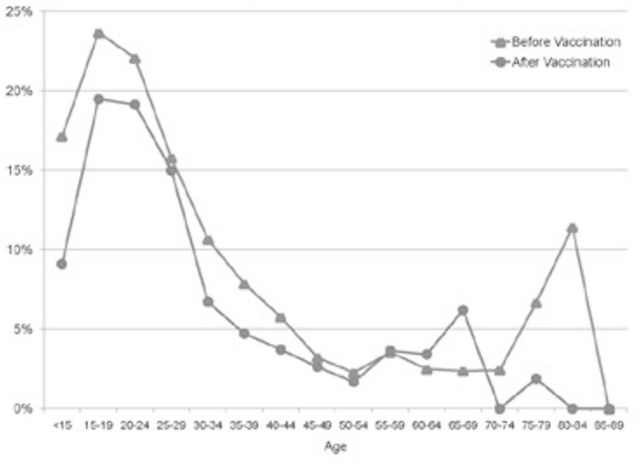
Background: The first HPV vaccine, the tetravalent Gardasil® designed to protect against HPV 6, 11, 16, 18 was approved by the FDA on June 8 2006. The vaccine was originally indicated for use in women aged 9-26, but has also been shown to be beneficial in susceptible women aged 24-45. The aim of this study was to assess any changes in HPV genotype distribution before and after the introduction of HPV vaccination.

Design: All cases that had HPV genotyping performed by a PCR-based method using the MY09/11 consensus primers and typing by RFLP from 04/01/2001 to 12/31/2008 were retrieved together with the age of the woman and corresponding Pap test diagnosis. The frequency of HPV 6, 11, 16, 18 in tests performed before and after 07/01/2006 were compared (pre and post-vaccination cohorts). Age and Pap test diagnosis which may have a significant impact on HPV prevalence and genotypes were controlled for statistically.

Results: 24411 HPV tests were performed in this interval; 12866 in the pre-vaccination cohort and 11545 in the post-vaccination cohort. The mean age was higher in the post-vaccination cohort (35.6 vs 38.4 years, p<0.001). The majority of HPV tests were performed on women with ASC-US. Overall there was a significant reduction of the rate of HPV 6,11,16,18-positive cases detected in the post vaccination cohort.

	Pre-Vaccination	Post-Vaccination	p Value
Any HPV	3904/12866, 30%	3161/11545, 27%	<0.001
HPV6	307/3904, 8%	183/3161, 6%	0.001
HPV11	60/3904, 1.5%	35/3161, 1%	0.12
HPV16	905/3904, 23%	571/3161, 18%	<0.001
HPV18	214/3904, 5.5%	151/3161, 5%	0.18

The differences in the HPV 6,11,16,18+ rate were significantly higher in the younger age groups, targeted by the vaccination.



The lower rate of HPV6,11,16,18 in the post-vaccination cohort persisted when controlling for age and Pap test diagnosis.

Conclusions: The significant decrease of the HPV types targeted by the tetravalent HPV vaccine in this study indicates the efficacy of the vaccination strategy.

401 Cytopathologic Characteristics of Papillary Thyroid Carcinoma with *RET/PTC* Rearrangement

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Background: *RET/PTC* gene rearrangement is found in a significant number of sporadic papillary carcinoma (PTC). *RET/PTC* rearranged PTC typically show classic PTC histology and present in younger patients with lymph node metastasis. With incorporation of molecular testing as an adjunct to thyroid fine needle aspiration (FNA) diagnosis, *RET/PTC* rearranged PTC is detected in cytology cases. In this study, we evaluated the cytopathologic parameters of *RET/PTC* rearranged PTC.

Design: Cytology specimens of histologically proven PTC were selected from the cytology files dating from April 2007 to June 2009. At the time of the FNA, cytology samples were collected for direct smears, thin-layer processing, and molecular studies. The material for the molecular studies was collected directly into nucleic acid preservative solution and *RT-PCR* was performed to amplify the fusion points of *RET/PTC1* and *RET/PTC3*. The cytology diagnoses were categorized according to the Bethesda system and were made independently of the molecular results. For each case, the original cytology diagnoses and the cytologic features (in particular, the number of intranuclear pseudoinclusions (IPI) in the most diagnostic area of 50 cells) were evaluated. The group of *RET/PTC* rearranged PTC was compared to a control group of *RET/PTC* negative cytology cases with histologically proven PTC in the resection specimen.

Results: Seven (7) cases of histologically proven PTC cases with *RET/PTC* rearrangement were identified. All seven cases were diagnosed as PTC cytologically and demonstrated an average of 3.2 IPI in the most diagnostic area. In contrast, only 1 of 8 cases of histologically proven PTC without genetic alteration were diagnosed as PTC on cytology and demonstrated an average of 1.0 IPI in the most diagnostic area. The differences in the 2 groups regarding the original diagnosis (p=0.001, Fisher's exact test) and IPI (p=0.004, T-test, parametric) were statistically significant.

Conclusions: Cytology specimens of PTC with *RET/PTC* rearrangement demonstrate an increased number of IPI and are associated with a larger proportion of cases diagnosed as PTC by cytomorphology alone when compared to PTC cases without this genetic alteration.

402 The Bethesda Thyroid FNA Classification System Versus the Old School Approach: Have We Really Improved Anything?

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Background: Our institutional early adoption of the Bethesda Thyroid Fine Needle Aspiration (FNA) Classification System is compared to our previously utilized diagnostic terminology and an operating characteristic is calculated.

Design: A total of 5114 thyroid FNAs were reviewed. 3207 FNAs from 2468 patients were retrieved from the archive of 2008; the diagnoses were reported based on the recent proposed Bethesda Classification System for reporting thyroid FNA. As a control group, 1907 FNAs from 1493 patients were retrieved from the archive of 2007; the diagnoses were reported according to individual pathologists' preference. We compared the frequency distribution and the histologic follow up of the diagnostic categories before and after the adoption of the Bethesda Classification System for reporting thyroid FNA.

Results: The specificities of the Bethesda System as a screening test for neoplasm and as a diagnostic test for malignancy were 68% and 93% respectively. The negative predictive values (NPV) of Bethesda for screening for neoplasm and diagnosing malignancy were 91% and 100% respectively. The specificities of non-Bethesda terminology as a screening test for neoplasm and as a diagnostic test for malignancy were 61% and 86% respectively. The NPVs of non-Bethesda for screening for neoplasm and diagnosing malignancy were 50%% and 56% respectively. Due to the limited number of negative cases with histologic follow-up, sensitivity and positive predictive value cannot be accurately estimated.

CYTOLOGIC-HISTOLOGIC CORRELATION

	HISTOLOGIC FOLLOW-UP (2007/2008)				
CYTOLOGIC CATEGORY	MNG & HT	FA	CA	TOTAL	TOTAL
Unsatisfactory	18/9	1/8	5/8	24/25	50
Negative for Malignancy	48/61	13/13	30/8	91/82	173
Indeterminate	NA/7	NA/7	NA/13	NA/27	27
Suspicious for Follicular Neoplasm	8/NA	11/NA	5/NA	34/NA	34
Follicular/ Hürthle Cell Neoplasm	14/33	25/34	15/35	54/102	156
Suspicious for Malignancy	3/2	1/2	6/26	10/30	40
Malignancy	2/0	2/0	46/112	50/112	162
TOTAL	93/112	53/64	107/202	263/378	641

MNG-multinodular goiter, HT-Hashimoto thyroiditis, FA-follicular/ Hürthle Cell neoplasm, CA-malignant neoplasm

Conclusions: Our findings suggest that there is no statistically significant difference in the distribution of diagnostic categories before and after the adopotion of the Bethesda System. However, the use of the Bethesda Thyroid FNA classification system significantly improves our ability to diagnose and triage thyroid malignancies and neoplasms preoperatively.

403 Subclinical Human Papillomavirus Infections Are Common among Women under Thirty Years of Age

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Background: The aim of this study was to conduct a thorough survey of the range and frequency of human papillomavirus (HPV) infections in young women with normal cervical cytology. These data are required for understanding HPV natural history and the wider context of clinical HPV screening, and for substantiating the ages at which exposure to high-risk (hr) HPV infections occurs.

Design: Cervical cytology samples diagnosed as negative for an intraepithelial lesion or malignancy (NILM) were collected from 214 women aged 13-19, 166 women aged 20-24, and 186 women aged 25-29 years. Purified DNA extracts were initially screened for HPV by PCR using GP5+/6+ and PGMY09/11 primers. Samples that tested HPV negative by these assays were also screened using FAP59/64 primers. HPV genotypes were identified by dot blot hybridization (GP5+/6+ assay) and/or by cycle sequencing (PGMY09/11 and FAP59/64 assays).

Results: Table 1 summarizes the findings.

Table 1

Age Range in Years	13-19	20-24	25-29
Number of Patients	214	166	186
Median Age	17.00	22.00	27.00
Any HPV Type Positive	85[1](39.7%)	88[2] (53.0%)	58[3] (31.2%)
High-Risk (hr) HPV∗ Positive	49[1'] (22.9%)	57[2'] (34.3%)	32[3'] (17.2%)
HPV16 or 18 Positive	31 (14.5%)	38 (22.9%)	20 (10.6%)

[1]27 different types detected; [2]36 types; [3]25 types; ∗HPV16, 18, 31, 33, 35, 39, 45, 51, 52, 58, 59, 66, 68, 73 or 82; [1'] 10 different hr types detected; [2'] 14 hr types; [3'] 8 hr types

In total, 45 different HPV types were detected among 231/566 (40.8%) HPV positive NILM patients. 'Any HPV', 'hrHPV', and 'HPV16 or 18', were each significantly more common in the '20-24' age group than in the '13-19' or '25-29' age groups (P<0.02, P<0.02, P<0.05 respectively).

Conclusions: These data demonstrate that subclinical HPV infections are extremely common in young women, especially in the 20-24 age group, 53.0% of whom were HPV positive with 34.3% being positive for hrHPV types. In all age groups, HPV16 or 18 were the most common hrHPVs supporting the implementation of current HPV vaccines at an early age. The exposure of adolescents to a wide range of other hrHPVs supports the development of additional vaccines. The findings are consistent with limiting negative cytology hrHPV testing primarily to women ≥30, with the detection of persistent hrHPV infections as an aid for identifying lesions of histopathological significance, and with the development of novel markers of cervical neoplasia.

404 Further Classification of Epithelial Lesions of the Salivary Gland

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Background: Fine needle aspiration (FNA) is beneficial in planning for surgical excision of salivary gland neoplasms. Certain cases are unable to be definitively diagnosed by aspiration due to technical insufficiencies, overlap in cytology and the necessity to examine the histopathology of the excised lesion. Indeterminate cases represent both benign and malignant lesions. Further classification of salivary gland lesions at FNA may allow better surgical management.

Design: A search of the laboratory information system from January 1990 to June 2009 identified 19 salivary FNAs signed out as an indeterminate epithelial lesions. All had surgical excision. Slides from these cases were distributed to 5 experienced cytopathologists who were blinded to the final diagnosis for their retrospective interpretation, including assessment of malignancy (benign, indeterminate, malignant), and further classification if possible with important criteria.

Results: The 19 cases identified included 13 malignant lesions (metastatic squamous carcinoma, 3; mucoepidermoid carcinoma, low grade, 2; mucoepidermoid carcinoma, high grade, 1; salivary duct carcinoma, 1; adenosquamous carcinoma, 1; adenoid cystic carcinoma, high grade, solid type, 1; carcinoma ex-pleomorphic adenoma in situ, 1; basal cell adenocarcinoma, 1; polymorphous low grade adenocarcinoma, 1; acinic cell carcinoma, 1) and 6 benign lesions (chronic sialadenitis, 2; pleomorphic adenoma, 1; Warthin's tumor, 1; basal cell adenoma 1; multinodular oncocytic hyperplasia, 1). 3 of 6 benign lesions were consistently called benign by all reviewers. 6 of 13 malignant lesions were consistently called malignant. No individual criteria were important in distinguishing these cases.

Conclusions: Indeterminate salivary gland FNAs are relatively uncommon, reflecting the well established cytologic criteria. Although pathologists should attempt precise classification of all salivary gland FNAs, it is important to recognize that a subset of cases remain impossible to classify by FNA alone. The following points were noted on review: 1. Indeterminate cases may represent either benign or malignant lesions and the latter may be either primary or metastatic. Primary neoplasms were a spectrum of lesions and morphologies. 2. Indeterminate lesions are more likely to be malignant on follow-up. 3. A subset of indeterminate cases was reliably categorized as benign or malignant on review, thus this categorization should be done, if possible, in otherwise indeterminate cases.

405 Reflex High Risk HPV Testing (HR HPV) Is Not Useful in Women with Atypical Squamous Cells – Can Not Exclude High Grade Squamous Intraepithelial Lesion (ASC-H)

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Background: ASC-H is a category created in the Bethesda 2001 and encompasses cells lacking the sufficient diagnostic features of HSIL. Current guidelines recommend immediate colposcopy with biopsy for follow up in patients with ASC-H. Recently a few small studies suggest that HR HPV DNA testing may also be used to triage patients with ASC-H. The aim of this study is to investigate if HR HPV testing in the ASC-H population identifies women at higher risk for high grade cervical neoplasia.

Design: We retrospectively identified all patients with a cervical cytology diagnosis of ASC-H within the consecutive 36 months between 2005 and 2007. Age, HR HPV DNA test results, and follow up cervical biopsy data were collected.

Results: During our study period, 192 patients with ASC-H (0.2%) were reported from a total of 92,111 Pap tests performed. The age of the patients ranged from 18 to 84 y (mean 34.2 y). From a total of 192 cases, 121 received follow up biopsy (63%). Eighty-two had reflex HR HPV testing (43%), and out of this group there were 53 with HR HPV testing and follow up biopsy (28%). Seven out of 12 patients who had ASC-H on Pap smear and were negative for HR HPV had HSIL on their biopsy (mean age 28.1 y). The positive predictive value of HR HPV positive in ASC-H cases for detection of HSIL is 68%. The negative predictive value of HR HPV negative for the absence of HSIL on biopsy is 42%. Sensitivity of the HR HPV test is 80% and specificity is 28% in ASC-H.

Table 1.Correlation of Follow Up Biopsy with HR HPV Results for ASC-H Pap Result

	Findings on Surgical Biopsy		
HR HPV Results (N)	**Negative**	**LSIL**	**HSIL**
HR HPV + (41)	3 (7.3%)	10 (24.4%)	28 (68.3%)
HR HPV - (12)	3 (25%)	2 (17%)	7 (58%)
Total (53)	6 (11%)	12 (23%)	35 (66%)

N = number

Table 2. Correlation of Surgical Biopsy and HR HPV testing

	Biopsy Diagnosis		
	LSIL and No Dysplasia	**HSIL and greater**	**Total**
HR HPV +	13 (32%)	28 (68%)	41
HR HPV -	5 (42%)	7 (58%)	12
Total	18 (34%)	35 (66%)	53

Conclusions: While HR HPV testing may further triage ASC-H into low and high risk categories for progression to high grade cervical lesions, a significant proportion of ASC-H women (20%) with high grade lesions were negative for HR HPV in our study. A higher than expected false negative rate and lower than expected negative predictive value were noted. Thus, our findings support direct colposcopy and biopsy instead of triage of an ASC-H result with HR HPV DNA testing.

406 Age-Specific Histologic Follow-Up Findings for Women with High-Risk Human Papillomavirus – Positive "Low-Grade Squamous Intraelithelial Lesion" (LSIL) Papanicolaou Test Results

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Background: While most women with LSIL Papanicolaou test (PT) diagnoses are managed by immediate colposcopy, the ASCCP recently recommended the option to manage postmenopausal women with oncogenic (high-risk) HPV (hrHPV) triage. The aim of this study was to determine the age-specific likelihood of CIN2/3 in follow-up biopsies of women with hrHPV+ LSIL and further edvaulate the use of HPV16/18 genotyping in LSIL.

Design: All women with LSIL PT diagnosed from 04/01/2001 to 12/31/2008 with concomitant hrHPV-positive results who had histologic follow-up (cervical biopsy, cone/LEEP or hysterectomy) within 6 months of the index PT were identified through searches of the computerized databases of our institution, which serves a low-risk population. PCR-based HPV DNA testing was performed using the MY09/11 primers with genotyping by RFLP. HPV types 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, and 68, included in the hc2 test were considered hrHPV.

Results: Of the 307 women with hrHPV+ LSIL results, 181 women aged 15-77 (mean 30+/-11) had follow-up histologic results (biopsy rate 59%). The biopsy rate was similar for women <30 or >=30 (113/195, 58% vs. 68/112, 60.7%, p=0.7). Follow-up biopsies showed 70 CIN1 (38.7%), 30 CIN2 (16.6%) and 20 CIN3 (11.1%) results. While the rate of CIN1 diagnoses did not differ with age, the rate of CIN2/3 diagnoses was significantly lower in women ≥30 (32.7% vs. 19.1%, p=0.047).

Age	Number	% CIN I	% CIN2/3
<20	24	37.5	37.5
20-29	89	40.5	31.5
30-39	38	39.5	23.7
40-49	23	34.8	13.0
50-59	6	33.3	16.7
>60	1	0	0
all hrHPV+ <30	113	39.8	32.7
all hrHPV+ ≥30	68	36.8	19.1

However, there was no difference between the rates of CIN2/3 in HPV16/18 women <30 and ≥30 (38.6% vs. 33.3%, p=0.62).

Age group	Number	%CIN I	%CIN2
HPV16/18+ <30	70	37.1	38.6
HPV16/18+ ≥30	30	33.3	33.3

These differences were caused by higher rates of HPV16/18 in women <30 with LSIL as compared to women ≥30.

Conclusions: We found a significantly lower rate of CIN2/3 in follow-up biopsies of women ≥30 with hrHPV+ LSIL, explained by a lower prevalence of HPV16/18 in these women as compared to women <30. Our results suggest a role for HPV genotyping in women ≥30 with LSIL, since the presence of HPV16/18 appears to confer a similarly high risk of follow-up diagnoses of CIN2/3 as in younger women.

407 Is Isthmic-Vaginal Cytology Smear Effective in Detecting Post-Radical Trachelectomy Recurrence of Cervical Cancer?

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Background: Radical trachelectomy is a fertility-preserving alternative for patients with early cervical cancer. According to our local, previously described protocol, postoperative follow up includes isthmic-vaginal cytology smear (IVCS) scheduled every 3 months for 2 years, every 6 months for 3 years, and then yearly. The aim of this study is to assess the role of cytology in early detecting of recurrence.

Design: A total of 94 patients treated with radical trachelectomy (1994 – 2007) with pertinent pathological, clinical and follow up data were identified from our prospective database. Within the follow up period, 913 IVCS were generated. These were evaluated by a group of cytopathologists.

Results: There were 1 – 27 smears per patient (median = 10). Seventy of the 94 patients (74.5%) had at least one abnormal IVCS, of which 45.7% had initial positive smears that subsequently converted to negative. Abnormal results included ASC-US (40.2%), AGC (41.9%), LSIL (11%), HSIL (4.6%), ASC-H (0.9%) and positive for malignant cells (1.4%). Within the follow up period of 1 – 149 months (median = 51 months), the only 2 central recurrences were successfully diagnosed by smears. One patient had no clinical findings except for an HSIL + AGC IVCS that was followed, 2 months later, by a cone biopsy confirming recurrence. The other had abnormal cervix and an IVCS reported as adenocarcinoma, confirmed by a concurrent biopsy. Pelvic (non-central) recurrence detected by pelvic CT occurred in 3 additional patients (IVCS was negative in 2 and HSIL in one). Although the negative predictive value in this series was 100%, the percentage of abnormal IVCS was high, mostly due to lower uterine segment endometrial cells and reactive postoperative changes.

Conclusions: This study confirms the role of IVCS in the early detection of central recurrence. More effective triage strategies are needed to identify the minority of women who will likely recur centrally to avoid unnecessary anxiety and the additional follow-up testing caused by the high percentage of abnormal results. The available evidence does, however, suggest that IVCS has a very low positive predictive value when used as a screening tool in the absence of symptoms or clinical signs.

408 Endoscopic Ultrasound-Guided Fine-Needle Aspiration (EUS-Guided FNA) of Pancreatic Metastases: A Study of 20 Cases

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Background: Metastatic lesions involving the pancreas are uncommon causes of pancreatic masses, but are important to recognize. The purpose of this study is to describe the cytomorphology, pitfalls, and ancillary studies of metastases in the pancreas diagnosed by EUS-guided FNA.

Design: We found a total of 1172 cases of pancreatic EUS-FNAs over a 4-year period, in which 20 cases (2%) had a confirmed diagnosis of a pancreatic metastasis. We reviewed the final cytologic diagnosis, ancillary studies, corresponding histological material, and clinical follow-up.

Results: The 20 metastatic cases included 10 (50%) cases of renal carcinoma,3 cases of malignant melanoma, and one case each of prostatic adenocarcinoma, breast carcinoma, renal leiomyosarcoma, gastric adenocarcinoma, esophageal adenocarcinoma, lung adenocarcinoma, and small cell carcinoma of lung. The average age of the patients was 61 years (range: 40-80 years). There were 11 females and 9 males. Seventeen cases (85%) had a previous diagnosis of malignancy of the same type. The metastatic prostatic carcinoma, small cell carcinoma, and one of the renal cell carcinoma cases occurred in patients with no prior diagnosis of malignancy. The average mean time between the initial diagnosis of cancer and the development of a pancreatic metastasis was 5.5 yr. (range: 0–19 years). The majority of the metastases were solitary lesions (15 cases; 75%). The location of the metastases included: 7 cases in the pancreatic head, 6 cases in the tail, one in the body, two with multiple lesions, and four unavailable. Immunohistochemistry on cell block material was utilized in 17 cases (85%). Seven patients underwent surgical removal of the tumor (pancreatectomy). All cases were confirmed on histological follow up, immunohistochemical stains, or review of the previous histology.

Conclusions: Our data shows that metastatic lesions in the pancreas are rare, but often occur as solitary masses in patient's with a known history of malignancy and can be seen several years after the primary diagnosis of malignancy. It is important to consider the possibility of a metastatic lesion in the pancreas because it may require different management than a primary pancreatic tumor. The combination of detailed clinical history, imaging findings, and cytomorphological features, in addition to the use of immunohistochemistry, can help to confirm the diagnosis and decrease the need for further workup.

409 Use of p16 Immunocytochemistry in Fine Needle Aspiration Biopsies of Metastatic Head and Neck Squamous Cell Carcinoma

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Background: High-risk human papillomavirus (HPV) is recognized as an independent risk factor for a subset of head and neck squamous cell carcinomas (HNSCC). HPV-related HNSCC is most often localized to the oropharynx, where greater than 50% of the tumors are associated with HPV-16 infection. The initial presentation for 13% of HNSCC patients is a neck mass. A fine needle aspiration (FNA) is the standard tool used to confirm the diagnosis of metastatic HNSCC. Despite a thorough clinical and radiologic workup, the primary site remains unknown in up to two thirds of these patients. Expression of p16 has been described as a marker of transcriptionally active HPV infection, as well as an indicator of improved survival and response to therapy in HNSCC. We sought to determine the value of using p16 immunocytochemistry (ICC) on FNA biopsies of neck masses with metastatic HNSCC to identify the HPV-related site of origin, and potentially enhance therapeutic options.

Design: We reviewed 90 FNA biopsies of neck metastases from patients with HNSCC from the cytopathology files at our institution. Primary sites included 43 from the oropharynx (19 tonsil, 17 base of tongue, 7 oropharynx NOS) and 47 from non-oropharyngeal sites (27 oral cavity, 11 larynx, 4 skin, 3 hypopharynx, 2 nasopharynx). Papanicolaou-stained slides were directly subjected to ICC, using the p16 antibody (Dako) and LSAB methodology. Tumors were classified as either p16 positive (strong, diffuse nuclear and cytoplasmic staining) or negative.

Results: Twenty-seven of 90 (30%) cases expressed p16 by ICC. Twenty (74%) of these p16 positive cases were metastases from oropharyngeal primary sites. There was a significantly higher proportion of positive expression for p16 among patients with a primary oropharyngeal carcinoma (20 of 43; 47%) versus those whose primary tumor was non-oropharyngeal (7 of 47; 15%) (p=0.0013).

Conclusions: p16 is expressed in 30% of FNA biopsies of neck masses diagnostic for metastatic HNSCC and is a reliable indicator of metastases from the oropharynx. The use of p16 ICC to detect HPV-related HNSCC that present in the neck may provide important prognostic information and improved therapeutic options. Finally, Papanicolaou-stained slides from FNA samples of neck masses may be directly used for the evaluation of p16 expression.

410 Implementation of the Bethesda System for Reporting Thyroid Cytopathology (BSRTC) Has Little Impact on the Rate of Diagnostic Errors or the Rate of Frozen Sections Requested for Thyroid Lesions

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Background: The Bethesda System for reporting thyroid cytopathology (BSRTC) has been suggested to make the cytology report "concise, clear, consistent and clinically relevant". In this study, we assessed whether improvement in quality of reporting has an impact on the rate of frozen sections requested during surgery.

Design: The study included all patients who underwent thyroid fine needle aspiration (FNA) from April 2006 to April 2009. Before implementation of BSRTC, we used generic categories for all non-gynecologic cytology: unsatisfactory, benign, reactive, atypical, and suspicious or positive for malignancy with a generous use of free text. Diagnoses before implementation were reconstructed to fit BSRTC. The rates of frozen section requested during surgery before and after implementation of BSRTC were compared using Chi-square and Fisher's exact test. Differences were considered significant at $p < 0.05$.

Results: We received 1671 FNAs (957 before and 714 after implementation of BSRTC) from 1339 patients, with 256 FNAs obtained from more than one site and 86 follow-up FNAs. 301 patients (191 before and 110 after implementation) had surgical resection. The rate of surgery after diagnosis remained the same, 72% before vs. 76% after implementation. The rate of frozen section before, 13.6% (26/191) was not statistically different than after, 20% (22/110), implementation of BSRTC. Within cases that had frozen section, 27% (13/48) were incorrectly diagnosed on cytologic examination compared to 13% (6/48) frozen section examinations. The difference was not statistically significant. There was no difference in these errors before or after implementation of BSRTC.

Conclusions: Implementation of BSRTC improves the quality of reporting but has little impact on frozen section rates or diagnostic errors for cases that are subjected to intra-operative consultation.

411 Comparison of Expression of ER, PR and HER2 by Immunohistochemistry in Cytology FNA Block Preparations to Surgical Tissue Blocks in Breast Cancer Patients

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Background: Immunohistochemistry (IHC) is a routinely used method to determine estrogen receptor (ER), progesterone receptor (PR) and HER2 status in breast carcinomas. Hormonal status at the time of breast cancer diagnosis provides important prognostic information and is predictive of response to hormonal therapy. Several factors have been known to affect the expression of these biomarkers one of which is tissue fixation. In this retrospective study we compared ER, PR and HER2 expression in breast cancers using archival ethanol-fixed cellblock obtained by fine needle aspiration (FNA) with formalin fixed surgical tissue blocks.

Design: Forty archival cell block preparations from primary breast cancers obtained by FNA performed by a pathologist for palpable breast masses or by a radiologist for image guided FNA were analysed. Cell blocks from 24 patients with invasive breast

cancer were available for review. Twenty-two of twenty-four patients had surgical tissue for comparison. Alcohol fixed cell block samples with no previous IHC for biomarkers were stained for ER, PR and HER2.
Results: There was good correlation for alcohol fixed cell block samples for ER, PR and HER2 expression with surgical tissue block samples with a Spearman Rho 0.81 p=0.006 for ER; Spearman Rho 0.83, p=0.006 for PR and Spearman Rho 0.71, p=0.005 for HER2. All HER2 reported as 1+ or 2 + in cell block or surgical samples were not amplified by FISH. ER was discrepent in 2 cases, PR in 1 cases and HER2 in 2 cases.
Conclusions: The correlation for ER and PR was slightly better than HER2. Therefore, IHC for ER, PR and HER2 on cell block sample in breast cancer patients is a reliable method for biomarker assessment when sufficient cellular material in cell block is available for IHC staining. Discrepent cases may reflect tumor heterogeneity of biomarker expression and may not influence treatment decisions. Use of FNA, which samples a wider area of the tumor, may be a more accurate method for biomarker assessment in breast cancer patients. FNA is particularly valuable in metastatic setting when no other tissue is available for analysis.

412 Morphologic Pitfalls of Hashimoto's/Lymphocytic Thyroiditis on Fine Needle Aspiration and Strategies To Avoid Overdiagnosis

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Background: There has been an emphasis on identifying papillary carcinoma or lymphoma within a background of Hashimoto's/lymphocytic thyroiditis (H/LT). However, misdiagnosing a neoplasm on fine-needle aspiration (FNA) of a thyroid with only H/LT and how to avoid this pitfall have not been adequately addressed.
Design: This study aimed to identify specific cytomorphologic features that may lead to the overdiagnosis of neoplasm in a background of H/LT. We found, within a five year period, nine cases in which thyroid FNAs were classified as suspicious or positive for malignancy/neoplasm, but the subsequent thyroidectomy specimens showed only H/LT. The cytologic features of these nine cases (Group I) were compared to eight control cases (Group II) from the same time period, in which the thyroid FNAs were also diagnosed as "suspicious or positive", but the tumors were confirmed against a background of H/LT on the thyroidectomy specimens (2 follicular adenomas and 6 papillary carcinomas). FNAs from both groups were re-evaluated for cytologic features commonly referred to in the cytology literature that may facilitate a correct diagnosis.
Results: Features most commonly leading to an overdiagnosis of papillary carcinoma in Group I were: pale, powdery or open nuclear chromatin, occasional nuclear grooves or holes, high cellularity with a microfollicular pattern, and a paucity of background lymphocytes. One helpful feature for differentiating H/LT from neoplasms was the presence of lymphocytes intimately infiltrating follicular groups. This likely overlooked feature was noted in most cases, even when background lymphocytes were scanty. In contrast, FNAs of true papillary carcinoma in Group II tended to display multiple typical cytologic features of papillary carcinoma in multiple cell clusters. These clusters were either devoid of infiltrating lymphocytes, or displayed only rare lymphocytes at their periphery. A microfollicular pattern with paucity of background lymphocytes was the major pitfall in cases diagnosed as suspicious for follicular neoplasms.
Conclusions: Features suspicious for papillary or follicular neoplasm are often seen in FNA of H/LT, leading to unnecessary surgery. Awareness of this diagnostic pitfall and recognition of differentiating cytologic features should avert the misdiagnosis.

413 Use of the ThinPrep® Imager for Evaluation of Slides Stained Immunocytochemically with Cytoactiv®

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Background: In Germany the Cytoactiv® test is commonly used for detection of HPV L1 capsid protein in conventional pap smears as well LBC specimens, as a biomarker to predict disease outcome of HPV high risk associated early dysplastic squamous lesions (Griesser et al, AJCP, in Press).Briefly, HPV L1 capsid protein negative early dysplastic lesions are significantly more likely to progress to histologically confirmed CIN 3 lesions than are L1 positive cases.
Design: The aim of this study was to evaluate the ThinPrep® Imager for evaluation of imaged ThinPrep slides immunochemically restained with Cytoactiv. Slides analysed with the ThinPrep Imager (Hologic, Bedford, MA) exhibiting LSIL or HSIL of moderate dysplastic type were used for immunochemical detection of the HPV L1 capsid protein with Cytoactiv (Cytoimmun diagnostics, Pirmasens, Germany) using the following procedure: 1.) Analyse imaged ThinPrep slides and use those classified as atypical LSIL or HSIL of moderate dysplastic type, 2.) Remove cover slip and stain with Cytoactiv, using manufacturer's instruction, 3.) Place ThinPrep slides with the initial barcode ID on the reviewscope, use the relocation function to mark the initially identified 22 fields of view (FOV), 4.) Evaluate Cytoactiv´s nuclear stain.
Results: The number of Cytoactiv positive cells on single ThinPrep slides ranged from 67 positive cells to 1 positively stained cell nucleus, Cytoactiv´s cut off value for a positive test result. The number of HPV L1 positive FOV ranged from 22 to 1.
Conclusions: Hologic´s ThinPrep Imager can be efficiently used to evaluate the Cytoactiv stain to speed up the reading of the stained result(s). To use this procedure is time saving and therefore economical, since it´s only necessary to focus on the 22 FOV instead of all the 120 visual fields of a single ThinPrep slide to screen for a Cytoactiv positive test result.

414 Prognostic Significance of HPV L1 Capsid Protein Detection with Cytoactiv® in an International Multicenter Study of 3000 Early Dysplastic Lesions

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Background: Data from ALTS confirmed that HPV DNA testing is not a useful triage strategy in low grade SIL (LSIL) and that it is still unresolved if individual cases of cervical intraepithelial neoplasia grade 2 (CIN 2) represent biologically low grade or high grade lesions. We and others have shown the prognostic significance of HPV L1 capsid protein detection with Cytoactiv on HPV high risk (hrHPV) associated mild and moderate dysplastic lesions. (i.e. Griesser et al, AJCP, in Press) In short, hrHPV positive and HPV L1 capsid protein negative,early dysplastic lesions are significantly more likely to progress to histologically confirmed CIN 3 lesions than are L1 positive cases.
Design: The aims of this study are 1.) to validate the prognostic relevance of HPV L1 capsid protein detection for early dysplastic lesions. 2.) to evaluate the impact of different preparation techniques (conventional Pap smear versus FDA approved LBC) on the sensitivity of L1 detection and its prognostic significance.
Results: Until June 2008, study centers located in Germany, USA, Sweden, Italy, Switzerland, and Australia contributed 3000 randomly selected cases of HIV negative, non-pregnant, non HPV L1 vaccinated women reported as LSIL (internationally) or in Germany as group IIID with subclassification into mild (LSIL) or moderate (HSIL) dysplasia. Follow up will be until June 2010. 53-85% of the LSIL and 23-50% of the HSIL cases have been positive for L1 capsid protein detection.
Conclusions: L1 positive mild and moderate dysplasias, reflecting productive HPV infection, showed a low risk of progression. L1 negative early dysplastic lesions, as non-productive infections or precancerous lesions, showed a high progression rate, as high as expected for severe dysplasias / squamous carcinoma in-situ.

415 Urothelial Carcinoma Either Arising in the Renal Pelvis or Upper Urinary Tract: A Retrospective Analysis and Cytological-Histological Correlation of a Consecutive Series

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Background: Although cytology of washings from upper urinary tract are used for the early detection and surveillance of urothelial carcinoma from renal pelvis/upper tract, there is paucity of information on the outcomes of cytologic-histologic discrepancies. We obtained histologic follow-up on a series of upper tract urinary cytologic specimens from a single institution to determine the diagnostic discrepancy frequency and outcomes.
Design: We performed a retrospective analysis of 50 consecutive patients with urothelial carcinoma either arising in the renal pelvis or involving the renal pelvis/upper urinary tract.We searched the laboratory information system (LIS) (Copath plus) for cytologic examination of urinary samples/genitourinary washings of these patients. The cytology and histology reports were reviewed and follow-up was obtained.
Results: The cytology specimens included genitourinary washings, voided urine samples and instrumented urine specimens. Most of the patients had 2-3 cytologic specimens. Of the cases included in the study, 40 cases were high grade urothelial carcinoma and 10 cases were low grade urothelial carcinoma. The cytologic examination of high grade urothelial carcinoma was concordant with histologic results in 24 (60%) of cases,7 (17%) cases were negative or reactive atypical cells and 9 (22%) cases were classified as "atypical urothelial cells, not further classified" Among the low grade urothelial carcinomas, the cytologic examination was concordant in 3 (30%) of the cases. 1 case was reported as high grade urothelial carcinoma, 1 case was reactive while the remaining 5 (50%) cases were reported as atypical without further classification.
Conclusions: The sensitivity of GU washings to sample upper urinary tract, targeting the renal pelvis lesions is 60%. The cytological diagnosis of "atypical not further classified category" is rendered in a large percentage of cases, several of which were neoplastic on follow-up. It may be useful to classify these further on cytology as either favor reactive or neoplastic to improve specificity of this diagnostic modality. As expected**,** the concordance among cytologic examination and histologic examination is much higher in high grade urothelial carcinoma than in low grade urothelial carcinoma.

416 Thyroid Follicular Lesion of Undetermined Significance: A Single Center Experience

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Background: The Bethesda 2007 Thyroid Cytology Classification defines follicular lesion of undetermined significance as a heterogeneous category of cases that are not convincingly benign nor sufficiently atypical for a diagnosis of follicular neoplasm or suspicious for malignancy. Others are placed in this category because of non-specific patterns, or compromising factors such as low cellularity, poor fixation or obscuring elements. In our institution, we refer to these cases as "indeterminate", and they are further sub-classified into two: 1) Low cellularity with predominant microfollicular architecture and absence of colloid (INa) and, 2) Nuclear features not characteristic of benign lesions (nuclear atypia) (INb). We reviewed these indeterminate cases seen over a period of eighteen months to document the follow-up trend using this 2-tier classification.
Design: A search of the cytology records was performed for the period between January 2008 and June 2009. All thyroid FNA cases were reviewed and the ones diagnosed as indeterminate were identified. Correlating follow-up FNA and/or surgical pathology reports were reviewed. The percentage of cases showing a malignancy was calculated.
Results: One hundred and seventy one indeterminate cases were identified, representing 2.8% of the 6205 thyroid FNA cases examined during the time under review (104 INa, 64 INb and 3 with both features). Records of follow-up procedures were available in 104 (61%) cases. Of the 59 cases of INa with follow-up, 35 (59%) had repeat FNA and 24 (41%) had surgical resection. In contrast 73% of cases of INb had surgical resection while

only 27% had repeat FNA alone. Malignancy was identified in 20% of all indeterminate cases. This was disproportionately more in the INb (56%) compared to the INa (7%) cases. The benign diagnoses were predominantly hyperplastic nodules (goiter) in both groups, while Hashimoto's thyroiditis was more prevalent in the INb group.
Conclusions: A diagnosis of "low cellularity with predominant microfollicular pattern in the absence of colloid" (INa) does not carry the same implication as that of "nuclear features not diagnostic of benign lesions" (INb). The INb category needs a more aggressive follow up than the INa category and may justify an immediate referral for lobectomy. Despite the vague morphologic criteria for this diagnostic category, the indeterminate rate remains relatively low and falls within the NCI recommendation (<7%).

417 Diagnosis of Squamous Cell Carcinoma (SqCC) of Lung on Cytology Specimens in the Era of Novel Chemotherapeutic Agents

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Background: Advances in lung cancer therapy have included Bevacizumab or Pemetrexed in non-small cell lung carcinoma (NSCLC). Recent literature suggests both of these drugs not be used in SqCC, either due to reported complications or a lack of efficacy. FNA cytology or bronchial wash/brush specimens are commonly obtained specimens in lung tumors, but there is limited literature evaluating subtyping of NSCLC in this therapeutic context. The aim this study was to retrospectively examine the accuracy of cytology diagnosis of SqCC/ recognizing squamous component in NSCLC.
Design: Lung tumors with a diagnosis of SqCC or NSCLC with a squamous component on resected lung specimens or on tissue biopsies in which prior cytology was available were retrieved from the pathology files of a tertiary hospital from 2001-2009. Results of any immunocytochemical (ICC) stains performed on the cytology specimens were also summarized. The diagnosis on tissue specimens was considered the gold standard and compared to the prior cytology diagnosis.
Results: 130 surgical cases were retrieved including 116 cases of SqCC and 17 cases of carcinoma with a squamous component. 110 cases were diagnosed as malignancies on prior cytology as follows: SqCC 66 cases, NSCLC not otherwise specified 33 cases, NSCLC (non-squamous) 9 cases, and Small cell carcinoma (SmCC) 2 cases. 23 cases were non-diagnostic. ICC markers indicative of squamous differentiation (p63, or CK34BE12) performed in 2 cases were positive and both cases classified as SqCC. The cytological and histological diagnoses in the 11 cases of SqCC that were misclassified on cytology as non-squamous were listed in the following table 1.

Histological Dx	Cytological Dx	Case Number
Basaloid SqCC	Small cell carcinoma	2
Poorly differentiated SqCC	Mixed small cell/non-small cell carcinoma	1
Poorly differentiated SqCC	Adenocarcinoma	3
Poorly differentiated SqCC	Poorly differentiated adenocarcinoma	1
Adenosquamous carcinoma	Adenocarcinoma	3
Moderately differentiated SqCC	Adenocarcinoma	1

Dx: diagnosis

Conclusions: Only 60.0% of SqCC were accurately recognized on cytology specimens. 30.0% of cases were categorized as NSCLC without further qualification. Additionally 10% of cases (mixed NSCLC, poorly differentiated SqCC and basaloid SqCC) can pose difficulties in subtyping SqCC. There is a lack of consensus of application of a panel of ICC on cytologic samples of NSCLC and this study warrants further investigation towards optimizing a panel of ICC stains in this era of newer therapeutic chemotherapeutic agents.

418 DOG1 Antibody Demonstrates Higher Sensitivity Than KIT for Gastrointestinal Stromal Tumors in Cytology Cell Blocks

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Background: Gastrointestinal stromal tumors (GISTs) usually occur in the wall of the gastrointestinal tract and may be inaccessible to endoscopic mucosal biopsy. Initial tissue sampling is therefore often obtained by endoscopic ultrasound-guided fine needle aspiration (EUS-FNA). Accurate diagnosis is critical to guide neoadjuvant therapy and surgical approach. Although 95% of GISTs are positive for KIT by immunohistochemistry on surgical specimens, staining for KIT is sometimes focal, and the limited material in a cell block can misleadingly appear KIT-negative. Several recent studies have demonstrated the higher sensitivity of the antibody DOG1 in the diagnosis of GIST in surgical material. In this study, we compare KIT and DOG1 in the diagnosis of GIST in cytology specimens.
Design: In total, 52 FNA cases with cell blocks diagnosed as GIST were selected. Cases were included if (1) the diagnosis was confirmed on surgical material (41 cases), or (2) when no surgical material was available, the cell block was positive for KIT (7 cases), or (3) in KIT-negative cases, the cell block was morphologically typical of GIST and negative for SMA and desmin (4 cases). In addition, 44 FNA cases of other intraabdominal or retroperitoneal tumors in the differential diagnosis of GIST were examined.
Results:

Immunohistochemical Results for DOG1 and KIT in Cell Blocks

	Total	KIT+/DOG1+	KIT-/DOG1+	KIT+/DOG1-	KIT-/DOG1-
GIST	52	40 (77%)	12 (23%)	0	0
Leiomyosarcoma	25	0	1 (4%)	0	24 (96%)
Leiomyoma	8	0	0	0	8 (100%)
Schwannoma	7	0	0	0	7 (100%)
Solitary fibrous tumor	2	0	0	0	2 (100%)
Desmoid fibromatosis	1	0	0	0	1 (100%)
Endometrial stromal sarcoma	1	0	0	0	1 (100%)

DOG1 was positive in all 52 GIST FNA cases. All 23 GIST specimens obtained by percutaneous FNA were positive for KIT. In contrast, 12 out of 29 GISTs (41%) sampled by EUS-FNA were negative for KIT; 8 of these showed KIT positivity on the surgical excision, while 4 lacked corresponding surgical material.
Conclusions: This study demonstrates that DOG1 is a more sensitive marker for GIST than KIT on the limited material available in cell blocks, and its sensitivity does not depend on the method of performing the FNA. In contrast, KIT was markedly less sensitive in EUS-FNA samples than those obtained by percutaneous FNA. DOG1 is also highly specific, with only 1 out of 44 non-GIST cases showing weak focal staining.

419 Double EGFR Mutation in Pulmonary Adenocarcinoma Detected by ThinPrep® Preparation

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Background: Mutation on exon 21 and deletion on exon 19 are related to non-smokers and adenocarcinoma of the lung are expected to response for targeted therapy by gefitinib and erlotinib. We have been using ThinPrep®(Hologic Inc.) to combine morophology and gene mutational analysis. Recently, two cases have been highlighted to show double EGFR mutation.
Design: ThinPrep® preparation was made from adenocarcinoma of the lung(four cases of bronchial brushing and four cases of fresh tissue subjected to frozen section diagnosis). After confirming adenocarcinoma or bronchiole-alveolar carcinoma(BAC), the carcinoma cells in fixative by ThinPrep® were subjected to PCR and subsequent direct sequencing for the mutational analysis of EGFR exons 18-21.
Results: Six cases were of wild type without any mutations in exons 18,19,20,21. Two cases showed double mutations, cases #1 with delE746-A750 and L858R, and cases #2 E709K and G719A on exon 18.
Conclusions: Formalin-fixed paraffin embedded(FFPE) materials are well known to be appropriate samples for mutational analysis. Our present study has shown the cells in the fixative(methanol-based) for ThinPrep® can preserve multiple mutations in the same tumor. Multiple EGFR mutations in the same case are rare and account for 34cases /1272 cases (2.8%) in large series(SRL Inc.). Case #1 with two common mutations, delR746-A750 and L858R is expected to respond well for the targeted therapy.

420 Restrospective Evaluation of Instituted Standard Adequacy Criteria for On-Site Adequacy Assessment of Thyroid Fine Needle Aspiration (FNA)

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Background: Criteria for specimen adequacy of thyroid FNAs on permanent smears are still controversial. To our knowledge, there are no criteria set forth for on site assessment of adequacy performed on Diff-Quick (preliminary smears) which results in lack of uniformity among different pathologists. In 2006 we implemented the requirement of a minimum of 6 clusters, each consisting of 10 follicular cells or more as standard criteria for on site adequacy.
Design: A total of 1031 thyroid FNA performed between 07/2006 and 03/2009 with on site assessment performed based on the standard criteria were reviewed. Agreement between on site and final assessment of specimen adequacy, Distribution of cytologic diagnoses were evaluated. Further, cyto-histologic concordance (neoplasia vs. nonneoplasia) was assessed on 221 cases with surgical follow-up.
Results:

Agreement on specimen adequacy between on site and final assessment

Final	On site-adequate N (%)	On site-inadequate N (%)	Totals N (%)
Adequate	851 (99.8)	70 (39.3)	921 (89.3)
Inadequate	2 (0.2)	108 (60.7)	110 (10.7)
Totals	853 (100)	178 (100)	1031 (100)

Distribution of cytologic diagnosis and cyto-histologic concordance

Cytologic diagnosis	N	Surgical follow-up N (%)	Concordant case N (%)
Adequate	921	221 (24.0)	139 (62.9)
–Nonneoplasia	722	66 (9.1)	62 (93.9)
—NH	638	62	58 (93.5)
—LT	84	4	4 (100)
–FL/HL	116	87 (75.0)	21 (24.1)*
–Neoplasia	83	68 (81.9)	56 (82.3)
—FN/HN	29	19 (65.5)	14 (73.7)
—PTC	46	41 (89.1)	34 (82.9)
—others	8	8 (100)	8 (100)
Inadequate	110	11 (10)	-

NH: nodular hyperplasia; LT: lymphocytic thyroiditis; FL/HL: follicular/Hurthle cell lesions of undetermined significance; FN/HN: follicular/Hurthle cell neoplasm; PTC: papillary carcinoma; Others: medullary/anaplastic/squamous cell carcinoma+ lymphoma + parathyroid adenom; * neoplasia on surgical follow-up.

Conclusions: Implementing standard criteria for on site adequacy assessment of specimen adequacy resulted in: 1) 93.0% level of agreement on specimen adequacy between on site and final assessment. 2) Nondiagnostic rate of 10.7%, compatible with published literature. 3) Cyto-histologic concordant rate of 93.9% for non-neoplastic and 82.3% for neoplastic lesions, compatible with published literature. 4) Provided consistency among cytopathologist when compared with previously published data. 5) More effective communication and high satisfaction from utilizers of our on site services.

421 Minimizing the Diagnosis of Follicular Lesion of Undetermined Significance and Identifying Predictive Features for Neoplasia

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Background: The diagnosis of follicular lesion of undetermined significance is problematic and frequently results in unnecessary surgery. The objectives of this

study are: 1) assessing whether the undetermined diagnoses can be minimized by using standard morphologic criteria 2) identifying predictive features for neoplastic nodules.

Design: A retrospective blinded review was perofrmed by 4 pathologists who agreed upon standard morphologic criteria for the diagnosis. Images illustrating the criteria were circulated. The group reviewed a total of 123 thyroid FNAs with a previous undetermined diagnosis and surgical follow-up. The reviewers assessed a total of 25 features semi-quantatively and rendered a diagnosis. The latter were correlated with histologic diagnoses. Logistic regression was perofrmed to identify predictive features for neoplasia (including adenoma).

Results:

CORRELATION OF CYTOLOGIC AND HISTOLOGIC DIAGNOSES

	Histologic	Diagnosis				
Cytologic Diagnosis	Nonneoplasia	Follicular adenoma	Follicular carcinoma	Papillary carcinoma	Parathyroid adenoma	Total
Nonneoplasia	47	9	5	2	1	64
Undetermined	21	9	1	1	-	32
Follicular neoplasm	9	9	1	3	-	22
Papillary carcinoma	3	-	-	2	-	5
Total	80	27	7	8	1	123

PREDICTIVE CYTOLOGIC FEATURES FOR NEOPLASIA

Features	OR (95% CI)	P
Cellularity	1.73 (0.95, 3.15)	0.07
Syncytium	1.64 (1.11, 2.42)	0.01
Scattered microfollicules	1.82 (1.20, 2.75)	0.005
Follicles with scalloped borders	1.78 (1.02, 3.10)	0.04
Scant cytoplasm	2.08 (1.26, 3.44)	0.004
Irregular nuclear membranes	1.69 (1.00, 2.84)	0.05
Nuclear overlapping	1.47 (1.01, 2.16)	0.04
Coarse chromatin	2.56 (1.34, 4.97)	0.004
Honeycombing	0.59 (0.41, 0.85)	0.004
Colloid	0.49 (0.29, 0.81)	0.005
Histiocytes	0.57 (0.35, 0.94)	0.03

Conclusions: Using standard criteria to assess undetermined follicular lesions: 1) provides a better stratification of cytologic diagnoses and a diagnostic accuacy of 68.1% is achieved. 2) Neoplasia is positively correlated with cellularity and presence of syncytium, scattered microfollicles, follicles with scalloped borders, scant cytoplasm, irregular nuclear membranes, nuclear overlapping, and coarse chromatin. 3) Neoplasia is negatively correlated with presence of honeycombing, colloid and histiocyte.

422 Utility of the Rotex Needle for Thyroid Fine Needle Aspiration (FNA)

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Background: Ultrasound-guided FNA is commonly used for the assessment of thyroid nodules. While most FNAs use a conventional hollow needle, we report our experience using the Rotex needle, which employs an inner screw-shaped stylet within an outer cannula. The Rotex needle has been used for the percutaneous FNA of lung, liver, kidney, lymph node, and other sites. Its use in the assessment of thyroid nodules has not been published. The majority of thyroid FNAs at our institution are performed by interventional radiologists using the Rotex needle without intraoperative adequacy assessment.

Design: Thyroid FNA results from 1/1/2009 to 9/30/2009 were retrospectively analyzed. Data collected included location of procedure, type of biopsy device used (Rotex or hollow needle), number of sites biopsied, diagnosis, and histologic diagnosis for cases that had concurrent core biopsy or subsequent resection. FNA diagnoses were categorized as nondiagnostic, benign (nodular goiter, hyperplastic nodule, adenomatoid nodule, thyroiditis), (suspicious for, cannot exclude, indeterminate for, consistent with, favor) follicular neoplasm, (suspicious for, consistent with) papillary carcinoma, and other carcinoma.

Results: FNA diagnoses from 285 thyroid sites among 228 patients were available, 176 using Rotex needles and 109 with hollow needles. Results are shown in Table 1.

Table 1: Thyroid FNA Results

Cytologic Diagnosis	**Rotex**	**Conventional**
Nondiagnostic	13 (7.4%)	12 (11.0%)
Benign	105 (59.7%)	76 (69.7%)
Follicular Neoplasm	50 (28.4%)	16 (14.7%)
Papillary Carcinoma	7 (4.0%)	5 (4.6%)
Other Carcinoma	1 (0.6%)	0

Histologic diagnoses were available for 48 sites after Rotex FNA and are shown in Table 2.

Table 2: Rotex Cytologic and Histologic Diagnoses

	Histologic Diagnosis		
Cytologic Diagnosis	Benign	Follicular Adenoma	Papillary Carcinoma
Nondiagnostic	1	0	0
Benign	12	7	1[a]
Follicular Neoplasm	9	10	1[b]
Papillary Carcinoma	0	1	6

[a]0.9 cm papillary carcinoma next to larger benign nodule [b]4cm adenoma containing 0.5cm papillary carcinoma

Conclusions: Thyroid FNA with the Rotex needle (93% adequacy) compared favorably to conventional needle FNA (89% adequacy), with a higher rate of follicular neoplasm diagnosis (28% vs 15%), possibly due to the larger tissue fragments obtained with the Rotex needle. Additionally, the Rotex needle offers the advantages of ease of use, high echogeneity, a more concentrated sample requiring fewer slides, and no need for an aspiration device.

423 Neuroendocrine Ductal Carcinoma *In Situ* (NE-DCIS) of the Breast: Cytological Features of 32 Cases

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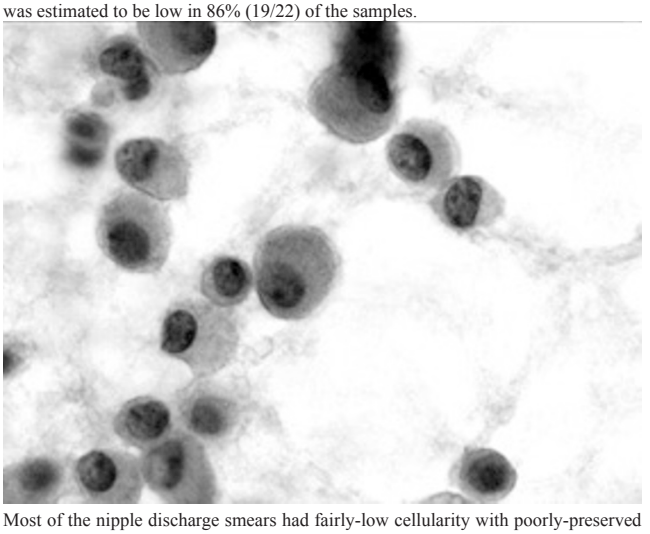
Background: Neuroendocrine ductal carcinoma *in situ* (NE-DCIS) of the breast has characteristic clinical and histological features and has been regarded as a distinct variant of DCIS. However, only a few case reports have described cytological findings of NE-DCIS in fine needle aspiration (FNA) smears. This study aimed to clarify the cytopathological profiles of NE-DCIS in a large series of FNA and nipple discharge smears.

Design: We cytologically analyzed 22 FNA smears and 17 nipple discharge smears obtained from 32 patients with NE-DCIS. All patients were Japanese (31 women and 1 man) with a mean age of 50.8 years. NE-DCIS was diagnosed using surgical specimens according to the histological criterion of >50% of cancer cells immunohistochemically expressing neuroendocrine markers (chromogranin A and/or synaptophysin).

Results: Backgrounds in FNA smears were clear (59%), mucoid (23%), hemorrhagic (14%) or necrotic (5%). Most FNA smears (95%) showed high cellularity. Characteristically, the NE-DCIS cells were loosely arranged in three-dimensional, solid clusters or singly dispersed. Well-developed vascular cores with or without cancer cells were occasionally recognized. Cancer cells were polygonal or spindle-shaped with a fine-granular, abundant cytoplasm. Nuclei with fine-granular chromatin were round or oval and often eccentrically located (plasmacytoid appearance) (Figure). Nuclear grade was estimated to be low in 86% (19/22) of the samples.



Most of the nipple discharge smears had fairly-low cellularity with poorly-preserved cell clusters in markedly hemorrhagic backgrounds, although two (12%) were extremely cellular with cytological characteristics similar to those of the FNA smears. Immunohistochemistry for neuroendocrine markers (chromogranin A and synaptophysin) confirmed the neuroendocrine nature of this tumor in adequate cytological specimens as well as histological specimens.

Conclusions: NE-DCIS of the breast has distinctive cytological features and can, therefore, be diagnosed as a neuroendocrine malignancy by most FNA and some nipple discharge smears.

424 Lab-on-a-Chip: The Future of Cervical Pre-Cancer Diagnostics

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Background: Cervical cancer development is directly linked to persistent infection with high-risk HPV and the expression of E6/E7 ongogenic mRNA transcripts. The aim of this project is to develop an automated point-of-care platform for sample preparation, nucleic acid extraction, amplification and detection of oncogenic HPV types. This study forms part of an EU 6th Framework funded project "Microactive" and is also supported by the CERVIVA Consortium, funded by the Health Research Board, Ireland.

Design: The point of care system consists of two biochips, one for sample preparation and the other for HPV detection, each involving a disposable chip and a reusable instrument. The HPV detection device allows on-chip parallel NASBA amplification and fluorescent detection of up to 16 different HPV mRNA types in 8 parallel microfluidic channels. The system has been tested on liquid based cervical cytology specimens from women with persistent oncogenic HPV infection.

Results: Studies on CaSki and HeLa cervical cancer cell lines showed that HPV mRNA could be detected down to the order of 5 cervical cells. Proof of concept experiments on clinical specimens (n=6) indicated that HPV mRNA was successfully extracted and amplified from clinical specimen using this point of care lab on a chip approach.

Conclusions: This project is the first to describe a complete lab on a chip system which successfully extracts nucleic acids sample preparation and detection on clinical specimens, ie cervical smears. The Microactive lab-on-a-chip system may be optimised further and adapted for a wide range of clinical tests involving biomarker detection.

425 Comparison of HPV DNA Detection Technologies; Hybrid Capture II (QIAGEN), Cervista™ HPV HR (Hologic UK Ltd) in a Northern Irish Screening Population

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Background: Cervical screening programmes worldwide are moving towards HPV DNA testing as part of the population screening process. We evaluated 2 HPV detection methods on ThinPrep specimens from a cervical screening population.

Design: Cervical smear specimens from 331 women were recruited through the Antrim Area Hospital, Antrim and the residual PreservCyt was used for HPV testing. Cytological diagnoses were made in accordance with UK National Health Service Cervical Screening Programme (NHSCSP). HPV DNA was detected by Hybrid Capture (hc2) for 13 high-risk HPV types and Cervista HPV HR (Cervista) for 14 high-risk types including HPV66 which is not included in the hc2 assay. The Cervista assay includes three different HPV specific mastermixes one of which (Mix 1) contains probes for HPV66, HPV56 and HPV51. HPV66 and 51 are the joint third most common HPV types in an all Ireland study of HPV prevalence.

Results: The prevalence of HPV was 23% by hc2 and 22% by Cervista. The concordance rate was 87%. A discordant result is a positive result by one assay and negative by the other test. Using detection of HPV in specimens with mild or greater abnormalities as the true positive, there was no significant difference in the sensitivity (P=0.148) or specificity (P=0.918) of the tests.

Conclusions: There was a high rate of concordance between the hc2 assay and Cervista despite the absence of HPV66 from the hc2 assay mix. This may be due to cross-reactivity of HPV66 with other HPV probes contained in the hc2 assay. There was no statistical difference in the sensitivity or specificity of either assay. Further studies containing greater numbers of cases with severe abnormalities will be performed. This study was sponsored by Hologic Inc. and forms part of the Irish Cervical Screening Research Consortium CERVIVA funded by the Health Research Board, Ireland.

426 The Value of a Duplicate Pap Smear Slide in Interpreting Atypical Squamous Cells, Cannot Exclude High-Grade Squamous Intraepithelial Lesion (ASC-H)

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Background: The category of ASC-H was added to the Bethesda system in 2001, which accounts for a small percentage of abnormal Pap smears. The ASC-H ratio is around 0.2-0.6% nationally and 0.4% in our laboratory. ASC-H encompasses a heterogeneous group of lesions including definite high-grade squamous intraepithelial lesion (HSIL), reactive metaplasia and benign hyperchromatic crowded groups. ASC-H comprises a higher number of high-grade lesions (30%-50%) compared to ASC-US (10%-15%). The treatment guidelines and follow-up procedures are different for HSIL, ASC-H and ASC-US; and a definitive diagnosis is crucial for proper clinical follow-up and treatment. This study aims to determine the role of evaluating duplicate slides in assessing ASC-H cases.

Design: 41 consecutive ASC-H cases were evaluated from October 2007 to March 2008. A duplicate Pap smear slide (DS) was prepared for each case. All DS were then reviewed blindly by a cytopathologist followed by a review of the corresponding histology. The DS were determined to be of value if HSIL or LSIL cells were identified upon review.

Results: 41 ASC-H DS were reviewed. Of these, 16 cases were diagnosed as HSIL or LSIL (13 HSIL, 2 LSIL vs. ASC-H, and 1 LSIL), 14 cases as ASC-H, 6 cases as ASC-US, and 4 cases as NILM. Therefore, reviewing DS potentially altered 16 ASC-H diagnoses to a more definitive interpretation. A total of 19 cases (19/41, 46.3%) had cervical biopsy follow-up, which revealed 10 cases of CIN2-3 (52.6%), 6 cases of CIN1 (31.6%), and 3 negatives (15.8%). Excluding the results of the DS review, the CIN 2-3 rate on ASC-H would only be 33.3%. 10 of the 16 cases with discrepancy had biopsy follow-up. 8 HSIL cases showed CIN 2-3 (6), CIN 1 (1), and negative (1); 1 LSIL vs. ASC-H case showed CIN 3; and 1 LSIL case showed CIN 1.

Conclusions: This study demonstrates that DS are very useful in evaluating ASC-H cases. Additional Pap smear material on the DS could substantially change the initial ASC-H diagnosis to HSIL or LSIL (39%); a change that would substantially alter patient management and clinical follow-up. HPV testing and p16INK4a immunohistochemistry have had some success in further classifying definite dysplastic cells and distinguishing reactive cells from ASC-H. However, these methods are not as cost effective as preparing a DS. Therefore, the value of DS should not be underestimated, and we highly recommend routine preparation and review of a DS before definitive interpretation of an ASC-H case.

427 Endoscopic Ultrasound-Guided Fine Needle Aspiration (EUS-FNA) of Pancreatic Cystic Lesions without Overt Cytologic Atypia: Proposed Diagnostic Categories with Utilization of Fluid Carcinoembryonic (CEA) Level

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Background: EUS-FNA is used to identify neoplastic pancreatic cysts that require resection. Particularly challenging are those lacking cytologic atypical/malignant epithelium. We reviewed our experience in EUS-FNA of pancreatic cystic lesions in an effort to identify ways to improve the diagnostic utility of this tool.

Design: We identified 247 pancreatic cystic lesions evaluated by EUS-FNA from the KU Medical Center cytology records for the period of 2002 to 2009. Twenty two (9 %) of these were resected. The initial cytologic diagnoses were inconsistent; however all cases lacked significant epithelial atypia. We retrospectively divided the cases into 3 categories: non-diagnostic, cyst contents only and cyst contents plus benign appearing neoplastic epithelium. The cytology diagnoses and the cyst fluid CEA levels were compared to the diagnoses on surgical excision.

Results: The patient population included 8 males and 14 females (age range of 21-80 years). The results are tabulated below.

EUS-FNA of pancreatic cystic lesions - Cytologic/histologic correlation

Cytologic Diagnostic category	**Non-Diagnostic**	**Cyst contents only, epithelium absent**		**Cyst contents and benign appearing neoplastic epithelium**	
Number	2	3 non-mucinous	7 mucinous	2 non-mucinous	6 mucinous
Cyst fluid CEA (ng/ml)	1 case > 800, 1 case ND	2 cases < 2, 1 case ND	5 cases > 800, 1 case < 2, 1 case ND	1 case > 800, 1 case ND	2 cases > 800, 2 cases > 400, 1 case < 10, 1 case ND
Histologic Diagnosis	2 MCNs	2 pseudocysts, 1 serous cystadenoma	4 MCNs, 1 IPMN, 2 non-neoplastic cysts[1]	2 serous cystadenomas	6 MCNs
Implication	Recommend repeat FNA, especially with high cyst fluid CEA	Identification of mucin and high cyst fluid CEA is highly predictive of a neoplasm		Identification of mucin and neoplastic epithelium with or without high cyst fluid CEA is highly predictive of a neoplasm	

D-not done, MCN-mucinous cystic neoplasm, IPMN-intraductal papillary mucinous neoplasm, GI-gastrointestinal. [1] Elevated CEA levels were only noted with the neoplastic cysts.

Conclusions: The proposed four diagnostic categories for pancreatic cystic lesions are easy to follow and have meaningful clinical implications when incorporated with the fluid CEA levels.

428 Galectin-3 and BRAF Mutation in Cases of Cytologically Suspicious of Papillary Carcinoma

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Background: Fine needle aspiration, known as the most accurate and cost-effective manner, still have some chances of indeterminate cases which are pauci-cellular and showing minor nuclear atypia, but most of are suspicious of papillary thyroid carcinoma (PTC). BRAF mutation was found about half of the PTC and galectin-3 as expressed by malignant tumor, helping us to differentiate malignancy from benign lesion.

Design: Subjected cases included 44 cases previously diagnosed as suspicious of PTC with cytologic examination which were confirmed with PTC after tissue examination and 4 benign controls. The subjected cases were analyzed the galectin-3 expression by immunohistochemical staining and the BRAF mutation by PCR-RFLP (Polymerase chain reaction-restriction fragment length polymorphism) with new restriction enzyme.

Results: All 44 cases of PTC and 2 out of 4 benign controls revealed galectin 3 expression. BRAF mutation was found in 9 cases of PTC only.

Prevalence of positivity of galectin-3 and BRAF mutation in cytology specimen

Category(n)	Galectin-3 (+)		Galectin-3 (-)	
	BRAF(+)	BRAF(-)	BRAF(+)	BRAF(-)
cPTC (40)	8	32	0	0
fvPTC (2)	0	2	0	0
dsPTC (1)	0	1	0	0
cmPTC (1)	1	0	0	0
Benign (18)	0	8	0	10

cPTC: conventional papillary thyroid carcinoma, fvPTC: follicular variant papillary thyroid carcinoma, dsPTC: diffuse sclerosing variant papillary thyroid carcinoma, cmPTC: cribriform morular variant papillary thyroid carcinoma

Statistical analysis of single and combined galectin-3 and BRAF mutation in cytology specimen

	Sensitivity	Specificity	Accuracy	PPV	NPV
BRAFV600E	20.4	100	43.5	100	33.9
Galectin-3	100	55.5	87	84.6	100
Galectin-3 and BRAFV600E	100	100	100	100	100
Galectin-3 or BRAFV600E	100	55.5	87	84.6	100

PPV; positive predictive value, NPV; negative predictive value

Conclusions: Assessment of galectin-3 expression demonstrates high sensitivity but low specificity. Evaluation of the BRAF mutation revealed high specificity and low sensitivity. This study suggests that the combination of the two methods in cytology diagnosis of PTC will be complementary.

429 Improved Detection of Malignancy in Pancreato-Biliary Ductal Brushing Specimens with ThinPrep Liquid-Based Cytology

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Background: To date, few studies have focused on the performance of ThinPrep (TP) versus conventional smears (CS) on biliary duct brushing specimens. In this study, we investigate: 1) whether TP shows enhanced sensitivity and/or specificity over CS in biliary ductal brushing specimens, and 2) the morphologic differences and potential artifacts of these two techniques.

Design: After IRB approval and patient consent was obtained at our institution, 24 patients with ductal abnormalities visualized on endoscopic retrograde cholangiopancreatography (ERCP) and/or computed tomography (CT) imaging were selected for hepatic, bile, or pancreatic duct brushing procedures. Two separate brushing specimens were obtained for each procedure: one brush sample was split and used to first make a CS then placed into liquid preservative and subsequently used to make a TP slide, and the other brush was placed directly into liquid preservative to make a TP slide. The order of brushing sequence was randomized and subsequent cytologic diagnosis made by a pathologist blinded to the order of sample collection. Sensitivity and specificity were calculated for TP versus CS, and comparison was made between the TP slides resulting from the split versus the non-split TP samples.

Results: Of the 24 patients involved, 19 were diagnosed with either pancreatic adenocarcinoma or cholangiocarcinoma (based on tumor resection and/or subsequent disease advancement), and 5 were diagnosed with benign disease. A total of 102

specimen slides generated from 65 procedures were obtained, and 100 were diagnosed as negative/atypical or suspicious/positive (with 2 CS being non-diagnostic). The sensitivity, specificity, PPV, and NPV of TP vs CS (TP/CS) were 47%/42%, 100%/100%, 100%/100%, and 42%/38%. The cellularity of TP slides was not affected by prior split to make a CS slide. Combining results of the 2 techniques did not increase sensitivity of TP.
Conclusions: TP shows superior sensitivity and NPV with identical specificity and PPV compared to CS in evaluating pancreato-biliary disease. CS preparations were hampered by obscuring blood, inflammation, mucous, and bile, and by occasional drying artifact. Performing both techniques is not necessary as CS results do not add diagnostic value to results obtained by TP. TP is a cost-effective, more easily processed (transporting fluid preservative vs slides) and superior method than CS in diagnosing pancreatobiliary malignancies.

430 Cytologic Parameters of Cervical Cytology Specimens Associated with Discordant "Equivocal" Hybrid Capture II High-Risk HPV DNA Tests Results: A Cytologic/Histologic Review of 191 Cases

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Background: The Hybrid Capture II (Qiagen) high-risk human papilloma virus (hrHPV) DNA test is an in vitro assay that performs qualitative and semiquantitative detection of hrHPV in cervical samples. Results are reported as a ratio of relative light units (RLUs) to a cutoff value (CO) or (RLU/CO), with a value of 1.0 separating negative from positive results. Per FDA labeling, values between 1.0 and 2.5 RLU/CO or "equivocal" specimens are retested and must show a value above 1.0 on retest to be resulted as a true positive specimen.
Design: We reviewed all hrHPV tests over a 27 month period at our institution, selecting 3 patient cohort subsets for corresponding cytologic and histologic comparison: a *concordant* "equivocal" group (1st hrHPV test between 1.0 and 5.0 RLU/CO with subsequent test positive), a *discordant* "equivocal" group (1st hrHPV test between 1.0 and 5.0 RLU/CO with subsequent test negative), and an unequivocal positive group (1st hrHPV test above 5.0 RLU/CO). We compared 46 ThinPrep pap tests from the *discordant* "equivocal" group with 29 ThinPrep pap tests each from the *concordant* "equivocal" and unequivocal positive groups. Finally, we examined available histologic follow-up corresponding to pap tests diagnosed as ASCUS in the *discordant* "equivocal" group.
Results: A total of 10,157 hrHPV tests were reviewed. Of these, 9395 (92.5%) were unequivocal positive or negative tests, 571 (5.6%) were *concordant* equivocal tests, and 191 (1.9%) were *discordant* equivocal hrHPV tests. Abundant blood and acute inflammation were associated with ThinPrep pap tests associated with an "equivocal" hrHPV test, with the *discordant* "equivocals" showing more profuse blood than *concordant* "equivocals" (p=0.014). Available follow-up histologic cervical biopsies from patients in the discordant "equivocal" group originally showing ASCUS cytology (n=12) were examined and showed high-grade CIN (CIN 2 or 3) in 25% of cases, and CIN 1 in 50% of cases.
Conclusions: Histologic follow-up from cervical cytology pap tests showing the most borderline of "equivocal" hrHPV tests (the *discordant* "equivocal" hrHPV test) reveals a highly significant degree of both low and high-grade dysplasia, suggesting that these cases are best managed as being unequivocally positive for hrHPV. The phenomenon of "equivocal" results in this cohort is most likely due to obscuring blood and inflammation in the cervical cytology specimens, and not lack of hrHPV.

431 Use of HMGA2 Overexpression by RT-PCR Performed on Suspicious Cytology Smears to Preoperatively Separate Benign from Malignant Thyroid Nodules

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Background: Follicular lesions continue to be a problem in thyroid cytopathology. No specific cytomorphologic features allow for distinction of benign from malignant follicular lesions. Approximately 40% of nodules that are diagnosed as "suspicious for follicular neoplasm" are resected. Up to 80% of indeterminate follicular nodules prove to be benign. Presently, there are no reliable ancillary tests to improve the preoperative detection of malignant follicular nodules. We sought to evaluate the performance of HMGA2 overexpression by RT-PCR performed on cells obtained from suspicious cytologic smears to distinguish benign from malignant follicular thyroid nodules.
Design: A search was performed to identify patients who had undergone thyroid FNA and subsequent thyroid nodule resection at our institution from 2001 to 2007. Only cases diagnosed as "suspicious" were included. Cases with 200 or more cells were included for HMGA2 testing. Resection specimen slides from the malignant cases were evaluated for architecture (solid, trabecular or insular), invasiveness (minimally vs. widely), extrathyroidal extension, necrosis, mitoses per 10 HPF, and presence of blood vessel invasion. HMGA2 expression was detected by one-step real-time qRT-PCR. HMGA2 mRNA expression was normalized to phosphoglycerate kinase mRNA expression and HMGA2 expression was expressed as relative fold change compared to HMGA2 expression in a thyroid cancer cell line (TPC-1).
Results: The following 164 patients were included: goiter 3 (1.8%), adenomatous nodule 15 (9.1%), follicular adenoma 60 (36.6%), Hurthle cell adenoma 27 (16.5%), follicular carcinoma 14 (8.5%), Hurthle cell carcinoma 11 (6.7%), papillary carcinoma (PTC) 5 (3%) and follicular variant of PTC 29 (17.7%). Using an HMGA2 expression ratio of ≥ 5.9 as a positive cutoff, the test had the following performance overall: sensitivity 71%, specificity 97%, PPV 94% and NPV 84%. HMGA2 overexpression had low sensitivity for Hurthle cell carcinoma (33%). When only non-Hurthle carcinomas including papillary and follicular carcinoma were considered, the sensitivity and specificity were 80% and 98% respectively.
Conclusions: HMGA2 overexpression can be performed on cells scraped from cytologic smears and it has high sensitivity and specificity for detecting malignant nodules in non-Hurthle cell carcinomas. HMGA2 overexpression analysis in conjunction with conventional cytology may markedly improve the ability to predict malignancy in follicular thyroid nodules.

432 Biliary Brushing Cytology: A Proposed Scoring System

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Background: Biliary Brushing cytology is a challenging area of histopathology. An objective, standardised scoring system is not established to complement traditional microscopic examination methods. A tabulated scoring system used as an adjunct to routine microscopic evaluation may be a helpful tool in distinguishing benign reactive from malignant specimens, particulary for trainee histopathologists or in low volume practices. The aim of this study was to develop and validate a simple standardised scoring system for liquid based biliary brushing cytology specimens as an adjunct to routine cytologic evaluation.
Design: Fifty consecutive biliary brushing cytology cases were retrieved. Each of eleven cytologic parameters (cohesion, architecture, chromatin quality, nuclear features, pleomorphism, cell borders, mitoses, nucleoli, pyknosis/karyorrhexis, nuclear/cytoplasmic ratio and nuclear preservation) were assessed and scored either 0,1,2 or 0,1 (mitoses) by one author. Each case was given a cumulative score. The minimum score per case was zero and the maximum 21. A score of ≥ 14 was regarded as positive and <14 as negative. Twenty-six cases had surgical follow-up, either biopsy, resection or both.
Results: The standardised scores of the 50 cases were compared with routine cytologic outcome (p=<0.001). Of the 26 surgical cases 19 had a malignant diagnosis. Thirteen of these had ascore of ≥ 14. Seven surgical specimens were negative for malignancy and all had a score of < 14. Significance of the score was assessed by a Fisher's exact test (p=0.005). Specificity = 100% and sensitivity =68.4%. These figures compare very favourably with conventional cytologic analysis.
Conclusions: A complementary standardised simple scoring system for biliary brushing cytology may be helpful as an adjunct to routine cytologic analysis, particularly for the pathologist-in-training or in low volume practices.

433 False Positives in Pancreatic Cytopathology: The Role of Pancreatic Intraepithelial Neoplasia and Acute Pancreatitis

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Background: Fine-needle aspiration (FNA) has played a significant role in the diagnosis of pancreatic masses. Overall sensitivity is between 76 and 91 percent while specificity ranges from 84 to 100 percent. To better understand the etiology of false positive diagnoses, we reviewed our experience with false positive cytologic diagnoses of pancreatic adenocarcinoma.
Design: The Anatomic Pathology files were searched for all FNAs of pancreas with subsequent resections. Cases with a diagnosis of positive for or suspicious for adenocarcinoma followed by a resection specimen not showing adenocarcinoma were reviewed and correlations between the surgical pathology and cytology findings were made.
Results: Five cases had a cytologic diagnosis of adenocarcinoma or suspicious for adenocarcinoma but resection specimens did not confirm the diagnoses. In two cases, a non-invasive intraductal papillary mucinous neoplasm (IPMN) without significant dysplasia was present with foci of pancreatic intraepithelial neoplasia (PanIN). In both cases, the degree of atypia within the IPMN was less than that seen cytologically. The nuclear features of the PanIN overlapped those seen in the smears interpreted as adenocarcinoma. In a single case, a neuroendocrine neoplasm was present accompanied by multifocal PanIN. The cytologic features of the neuroendocrine neoplasm did not correlate with the material cytologically diagnosed as high grade dysplasia or adenocarcinoma. The cytologically atypical ductal epithelium corresponded closely to that seen in the PanIN. Two diagnoses of adenocarcinoma were made cytologically but the pancreatectomy specimens revealed pancreatitis with reactive atypia of ductal epithelium. The marked atypia seen histologically in the ductal epithelium corresponded to epithelial cells cytologically misinterpreted as adenocarcinoma.
Conclusions: The cytologic diagnosis of high grade pancreatic adenocarcinoma has high specificity. Cytologic separation of well differentiated adenocarcinomas from other lesions is less accurate. Five cytologic misdiagnoses occurring in 105 cases undergoing pancreatectomy. These misdiagnoses correlated with histopathologic changes of intermediate to high grade PanIN or marked reactive atypia in a background of severe pancreatitis. While reactive atypia in the setting of chronic pancreatitis is well recognized as a potential cause of false positive cytology, PanIN may be an under recognized, but significant source of false positive results.

434 Fine Needle Aspiration of Chronic Sclerosing Sialadenitis (Kuttner's Tumour): A Potential Pitfall. A Series of 4 Cases

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Background: Chronic sclerosing sialadenitis (CSS) is a benign and chronic inflammatory condition of the salivary gland. Clinically, CSS patients may present with a neck nodule or mass often suggesting a neoplastic process. Fine needle aspiration (FNA) is frequently used to evaluate these lesions. We present a series of 4 cases of CSS. The cytologic as well as the tissue biopsy findings of these cases are reviewed.
Design: 4 cases, two females and two males, that underwent resections of neck nodules with a surgical pathology diagnosis of CSS were identified. The histopathology was reviewed by two pathologists. The clinical and pathologic findings were reviewed.
Results: The mean age was 66.2 years. All patients presented with a palpable lesion in the head and neck, 3 with submandibular nodules and one with a parotid nodule, measuring 1.5 cm in average size. Three patients had a history of a squamous cell carcinoma (SCCA) of the head and neck. A patient had a history of malignant melanoma

of the neck skin. All 4 cases had a FNA prior to surgery. The immediate diagnosis (IDx) given in 3 cases was carcinoma, SCCA, and salivary gland neoplasm respectively. The final diagnosis (FDx) in these cases was SCCA, basaloid neoplasm, and salivary gland neoplasm respectively. In a case, only a FDx of SCCA was given. Cytologically, all the cases showed rare cell clusters with squamoid appearance and tridimensional architecture and minimal to mild nuclear atypia. A cellular aspirate was not seen in these cytologic preparations. All the patients had intraoperative consultation (IOC). Grossly, 3 cases showed no evidence of tumor; and in one case, a 1.1 cm white tan nodule was identified. In the IOC, the lesions were identified as benign (n=2), atrophic salivary gland with chronic inflammation(n=1), and as sialadenitis with atypical glands (n=1). All the resected specimens show benign salivary glands with prominent fibrosis, encasing the lobules, focal squamous metaplasia, mild to moderate lymphoplasmacytic infiltrates, features which mirror the FNA findings. The diagnosis in these surgical specimens were consistent with CSS.

Conclusions: CSS is a potential pitfall in the FNA interpretation of salivary gland lesions. Awareness of this entity, adherence to strict cytologic criteria, and careful clinicopathologic correlation are helpful in preventing a misinterpretation.

435 Epidermal Growth Factor Receptor (EGFR) Mutations, KRAS Mutations, and EGFR Gene Amplification by FISH on Cytology Specimens

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Background: In patients with non-small cell carcinoma (NSCLC), the presence of a somatic EGFR mutation is significantly associated with response to gefitinib and erlotinib. NSCLC with KRAS mutation do not respond as well to anti-EGFR therapy. Patients with tumors having EGFR gene amplification (EGFR-GA) and/or high polysomy have shown favorable outcomes when treated with tyrosine kinase inhibitors. The experience of these tests in cytology specimens is limited.

Design: 34 consecutive patients with NSCLC that underwent EGFR mutation, KRAS mutation, and EGFR-GA by Fluorescent In Situ Hybridization (FISH) analysis on cytology specimens were studied. Genomic deoxyribonucleic acid (DNA) was extracted from fresh fluid, or formalin fixed paraffin embedded cell blocks (FFPE-CB). Polymerase chain reaction (PCR)-based Fluorescence Fragment Analysis Assays were performed for designated mutations in the Exons 19 and 21; and they were confirmed by direct cycle sequencing methods. The Exon 2 of the KRAS gene was amplified by PCR, and followed by direct nucleotide sequencing to evaluate point mutations. EGFR-GA FISH was studied with a standard method. Amplification was defined as EGFR gene to chromosome 7 ratio ≥ 2. High polysomy was defined as 4 or more copies in $\geq 40\%$ of the cells.

Results: The mean age of the patients was 62.7 years-old.17 males and 17 females were studied. 30 lung adenocarcinomas, 3 NSCLC, favor adenocarcinoma, and one squamous cell carcinoma were studied, including at total of 36 specimens; the specimen was a fine needle aspiration (FNA) in 21 cases (12 lung, 5 neck, 2 mediastinal,1 chest wall, and 1 abdominal wall), a pleural fluid in 12 cases; and a pericardial fluid, a bronchial wash, and a fresh cerebrospinal fluid (CSF), one each. FFPE-CB were studied in 35 specimens; and one fresh CSF was used. Respectively, 5 (13.8%) cases showed a somatic EGFR mutation, 4 in Exon 19 (15 base pair deletion) and one in Exon 21 (L858R). Four of these latter cases showed also EGFR-GA. 11 cases showed Exon 2 KRAS mutations. 1 case with G12A; 4, G12C; 1, G12R; 2, G12S; 2, G12V; and 1, G13C. Two of these cases showed EGFR-GA. 7 of the cases negative for EGFR and KRAS mutations showed EGFR-GA.

Conclusions: In our experience, routine clinical cytology specimens including fluids and cell blocks can be reliably used to perform EGFR-GA by FISH, and assay for KRAS, and EGFR mutations. Therefore, this testing may be useful in the patient's management.

436 Impact of a Location-Guided Imaging System on the Frequency Distribution of Diagnostic Categories in Gynecologic Cytology Using SurePath Preparation

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Background: Recently, the FDA has approved the use of the location-guided imaging system, FocalPoint GS, (Becton Dickinson, Burlington NC) on SurePath Pap tests (Becton Dickinson) for primary screening. The objective of the current study was to evaluate the impact of the use of the FocalPoint GS on the distribution frequency of diagnostic categories before and after implementation of the location-guided imaging system.

Design: A search of the laboratory information system (Co-Path) was performed to identify all SurePath Pap tests processed in our laboratory the first 4 months (from 5/09 to 9/09) after implementation of the location-guided imaging system. We also collected data from 5/08 to 9/08 on all SurePath Pap tests processed in our laboratory, as the control. During the period from 5/08 to 9/08, the FocalPoint Slide Profiler (Becton Dickinson) was used for imaging. The number of cases in each diagnostic category was obtained, percentages were calculated, and then compared. Diagnostic categories included the following: negative, ASCUS, LSIL, HSIL, ASC-H, AGC, positive for malignancy, and unsatisfactory.

Results: The results are summarized in TABLE 1. The use of the location-guided imaging system resulted in higher percentage of LSIL, ASCUS, ASC-H, and AGC. However, the difference was not statistically significant (2 tailed paired student t-test, $p>0.05$). The ASC to SIL ratio was 1.4 and 1.9, before and after implementation of the FocalPoint GS imaging system, respectively. Again, the difference was not statistically significant (z test, $p>0.05$).

Comparison of SurePath Pap Diagnoses Before and After Implementation of the FocalPoint GS System

	FocalPoint Slide Profiler		FocalPoint GS	
Diagnostic Category	**No. of Cases**	**Percentage**	**No. of Cases**	**Percentage**
Negative	25,977	91.5	23,621	89.1
ASCUS	1,244	4.4	1,695	6.4
LSIL	841	3.0	883	3.3
HSIL	59	0.2	57	0.2
ASC-H	52	0.2	83	0.3
AGC	19	0.1	45	0.2
Malignancy	6	0.0	1	0.0
Unsatisfactory	202	0.7	140	0.5
Total	28,400	100	26,525	100

Conclusions: In our laboratory, the use of the location-guided imaging system resulted in a small increase in the detection of LSIL, but not HSIL. In addition, there was an increase in the proportion of atypical categories with the use of the FocalPoint GS imaging system. The lack of statistically significant differences may be attributable to the low number of cases.

437 High Risk HPV in Normal Pap Smears: A Long Term Correlation between HPV Viral Load, Age, and Disease Progression

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Background: Human Papilloma virus infection (HPV) is a well established risk factor for cervical cancer. However, the association between HPV viral load and the degree of cytologic atypia remains controversial. Cytologically negative PAP tests that test positive for high risk HPV represent subclinical infection. It is known that older patients with HPV viral load are more likely to harbor subclinical infections. This study aims to establish a link between HPV viral load and age with subsequent detection of significant cervical lesions (cervical intraepithelial neoplasia 2 (CIN2) or higher).

Design: 776 of cases with initially normal PAP smears and positive high risk HPV (Hybrid Capture 2 HPV DIGENE) between January 2004 and December 2007 were collected from the Department of Pathology archives. The initial viral load was recorded as very low, low, moderate, and high. The cases were analyzed for initial viral load, age at initial PAP smear, and follow-up cytologic or surgical gynecologic specimens over a 2-5 year period. Cases with no cytologic or surgical follow-up were rejected. Patients were stratified as less than or greater than or equal to 30 years of age. A Z-test for two proportions analysis was performed to determine associations. A p value of less than 0.05 was considered significant.

Results: Results are summarized in tables 1 and 2. The difference between very low and moderate viral levels ($p=0.028$) and the difference between low and moderate viral load ($p = 0.0038$) was statistically significant. The difference between moderate and high viral loads was not statistically significant ($p=0.295$). The association between age and follow up abnormalities (47% versus 31%) had a two tailed p value < 0.001.

Viral load vs follow-up abnormalities

Initial viral load	Very low	Low	Moderate	High
Positive cases	17	180	69	5
Total cases	60	549	150	17
% cases	28%	33%	46%	30%

Age vs follow-up abnormalities

Age	<30	>30
Positive cases	111	167
Total # cases	238	538
% cases	47%	31%

Conclusions: Patients less than 30 years of age were more likely to have disease progression than patients greater than 30 years of age (p value < 0.001). An initial moderate HPV viral load is associated with a higher frequency of disease progression than low or very low viral loads ($p=0.028$, $p=0.0038$). There was no statistically significant difference between moderate and high viral loads in relation to disease progression, but the number of cases of high viral load was insufficient.

438 Echobrush Versus Standard EUS-Guided FNA of Cystic Lesions of the Pancreas. Preliminary Experience

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Background: Cystic lesions of the pancreas are being detected with increasing frequency. EUS-guided FNA (EUS-FNA) of cystic fluid and exfoliated cells is one of the most accurate method of diagnosis but still has limited sensitivity. A new, through-the-needle cytologic brush system (EchoBrush) has been approved for use during EUS evaluation of cystic lesions of the pancreas.

Design: Data from 105 EUS-FNA of pancreatic cystic were analyzed in order to compare the cytologic yield of EchoBrush compared with conventional EUS-FNA. An attending cytophatologist was present on site to assess specimen adequacy in all the cases. Diagnostic yield of both procedures, as well as EUS-FNA and EUS-EchoBrush related adverse events were recorded. Statistical analysis was performed with the SPSS 15.0 version.

Results: A total of 105 cystic lesions of the pancreas in 105 patients (37 men and 78 women, mean age of 62.7 ± 11.9 years) were included in the study. Mean size of lesions was 23.2 ± 18.6 cm. In 76 cases (72.4%) we obtain the samples through conventional EUS-FNA, whereas in 39 (37.1%) we used EchoBrush. Diagnostic material was obtained in 87.2% of cases using EchoBrush and in 68.3% with conventional EUS-FNA. Adequacy of the samples and diagnostic yield were higher using EchoBrush with $p=0.028$. There was not EUS-FNA either EUS-EchoBrush related complications.

Conclusions: This study suggests that cytological specimens from pancreatic cystic lesions obtained using EchoBrush at the time of EUS are superior to conventional EUS-FNA mainly because of the higher yield of epithelial cells. Larger studies are needed to compare both methods.

439 Implementation of Evidence-Based Immunostain Panels for Diagnosis of Pleural Effusions with Atypical Epithelioid Cells: Theory vs. Practice

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Background: Based on the incidence of carcinomas, cost-effectiveness of various immunostains and application of Bayesian statistics, we previously developed 3 evidence-based immunohistochemical (EB-IHC) panels for the diagnosis of pleural effusions (PE) with atypical epithelioid cells (AEC).

Design: The EB-IHC panel to distinguish benign from malignant PE included MOC31, BER-EP4, calretinin, and CK5/6. The panel for origin of tumor in the malignant PE included TTF-1, PSA, CDX2, calretinin for male and TTF-1, ER, CA125, and calretinin for female patients.The rationale for utilization of these panels was presented to our 5 cytopathologists and ordering the panels was facilitated by computer codes. Use of the panels was recommended but not mandated. One year later we assessed utilization and diagnostic applicability of the panels and reasons for non-compliance with their usage.

Results: 955 PE were diagnosed during the year. Immunostains were used in 122 PE with AEC (48 benign and 74 malignant). Malignant PE in females (N=46) included 16 breast, 10 lung, 9 mullerian, 3 neuroendocrine/SCC lung, 2 cervix, 1 case each of colon, stomach, duodenum/pancreas, bile duct, and 2 unknown primaries. Malignant PE in males (N=28) included 8 lung, 5 colon, 3 prostate, 3 neuroendocrine/SCC lung, 2 pancreas, 1 each of stomach, urothelium, renal cell, larynx, breast, mesothelioma, and unknown primary. The distribution of primaries was similar to that in the previous study. Complete EB-IHC panels were used in 16% (20) cases (15 benign, 5 malignant). Stains selected from the panels were used in different combinations in 21% (26) cases (13 benign, 13 malignant). In the remainder 62% (76) cases (20 benign, 56 malignant), 1 to 11 additional IHC (Add-IHC) were used per case. Use of Add-IHC identified the tumor origin in only 7 (9%) of these 76 cases (6 neuroendocrine/SCC and 1 renal cell carcinoma). The most frequently used Add-IHC that did not yield additional diagnostic information were CK7, CK20, WT-1, GCDFP-15, mammaglobin, and B72.3.

Conclusions: Reasons for poor compliance included limitations in EB-IHC panel design and reluctance of pathologists to modify their practice. A more pragmatic approach that combines clinical parameters, immunostains selected from the panels rather than sequential use of complete panels, targeted use of neuroendocrine markers, and an algorithm for diagnosis of PE with AEC with periodic reassessment of its clinical utility has been adopted.

440 Improving Negative Predictive Value of Endobronchial Ultrasound Guided Transbronchial Needle Aspiration (EBUS-TBNA) Staging of Mediastinal Lymph Nodes

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Background: Endobronchial ultrasound-guided transbronchial needle aspiration (EBUS-TBNA) is a well established and cost effective method to stage patients with thoracic cancers. Previously reported negative predictive values range between 76% and 86%.

Design: A retrospective study was performed to identify patients who underwent EBUS-TBNA for pre-operative staging of lung carcinoma or mesothelioma between October 2006 and July 2009 at a large cancer center. All cytology samples obtained by a single thoracic surgeon who is highly experienced in EBUS-TBNA were selected for review. All samples had immediate on-site assessment for adequacy by a senior cytotechnologist. Samples lacking lymphoid tissue were reported as non-diagnostic, rather than negative. The cytology diagnosis for each lymph node station was correlated with subsequent histology.

Results: Of 315 EBUS-TBNAs performed, 95 samples from 53 patients had subsequent surgical resection within 24 days. The underlying diagnosis was mesothelioma (47%) or non-small cell lung carcinoma (53%). The average size of sampled lymph nodes was 0.8 cm (range 0.3-2.1 cm). The most common lymph node stations to have histologic follow-up were stations 7, 4R, and 4L (92% of samples). Diagnostic material was obtained in 92 of 95 (97%) samples. The cytologic diagnoses for this group contained 8 false negative diagnoses (8%) and 1 false positive diagnosis (1%). The sensitivity, specificity, positive predictive value, and negative predictive value were 56%, 99%, 83%, and 91%, respectively. Unanticipated findings included: each of the 8 false negative results occurred in mesothelioma patients, the lymph node station that was most prone to a false negative was station 7 (7 of 8 samples), and the majority of false negative samples were reported to contain adequate evidence of lymph node sampling (7 of 8 samples).

Conclusions: The negative predictive value of EBUS-TBNA can approach that of mediastinoscopy through cooperation across medical disciplines. An experienced bronchoscopist is necessary to provide diagnostic samples even on lymph nodes less than 1 cm. An experienced cytotechnologist or pathologist is needed to quickly and accurately evaluate specimens for evidence of lymph node sampling and/or presence of malignant cells to provide immediate feedback during the procedure. Even with multidisciplinary collaboration false negative diagnoses occur, and appear to be a result of sampling rather than misinterpretation.

441 Human Papillomavirus DNA and mRNA Prevalence and Persistence in a Cohort of Human Immunodeficency Virus Positive Women in Ireland

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Background: Human papillomavirus (HPV) infection is necessary for cervical neoplasia and cancer development. HIV positive women are known to suffer increased prevalence of HPV infection, persistent HPV infection and thus increased cervical disease. With anti-retroviral therapy increasing the life expectancy of HIV positive women, the risk of developing cervical disease will also increase. In this study, we examined a HIV positive cohort of women for high-risk HPV (HR-HPV) DNA prevalence, HPV mRNA oncogenic expression and cytological status in relation to clinical and socio-demographic data. This study forms part of the CERVIVA research consortium funded by the Health Research Board, Ireland.

Design: 300 HIV positive women were recruited to the study through the Genitourinary and Infectious Disease Clinic at St James' Hospital, Dublin. PreservCyt™ cervical smears were taken at time of recruitment and at 12-18 month follow-up periods. Clinical data was recorded for each patient. Cytological diagnoses were made according to BSCC guidelines. HR-HPV DNA positivity was determined using Hybrid Capture II assay (Qiagen Ltd., UK); with HPV mRNA expression detected using the PreTect™ HPV Proofer assay (NorChip AS, Norway) for 5 HPV types (16, 18, 31, 33 and 45).

Results: We found a HR-HPV DNA prevalence of 49%, a HPV mRNA prevalence of 20% and an abnormal cytology rate of 25%. In women with cytological abnormalities HR-HPV prevalence was 88% and HPV mRNA prevalence was 40%. HPV 45 was the most common mRNA type found followed by HPV 18, 16 or 33 and 31. To date HR-HPV DNA rates were 53% (26/49) at baseline and 45% (22/49) at 12-18 month follow-up. HPV mRNA rates were 21% (10/48) at baseline and 23% (11/48) at follow-up. In women with CD4 counts (>200 x 106/L) HPV DNA and mRNA rates were 49% and 18% respectively with HPV DNA and mRNA rates in women with CD4 counts (<200 x 106/L) being 78% and 44%.

Conclusions: HPV mRNA prevalence of 20% is higher than normal populations but not unexpected due to the increased persistence of HPV infections in HIV positive women. There is a direct correlation between low CD4 counts (<200 x 106/L) and increased HPV DNA and mRNA positivity.

442 Fascin Stain as a Potential Marker of Invasiveness in Carcinomas of the Urinary Bladder: A Retrospective Study with Biopsy and Cytology Specimen Correlation

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Background: The biologic behavior and prognosis of urothelial carcinomas of the urinary bladder are largely dependent on the presence of invasion. Evaluation of invasion cannot be determined on cytology and can be challenging in biopsy cases with limited sampling. Recent studies of bladder resection specimens suggest that Fascin overexpression may be a marker of aggressive urothelial carcinomas and can help facilitate the assessment of invasion.

Design: We selected thirty-five patients with concurrent urine cytology and biopsy proven invasive urothelial carcinoma. Fascin immunohistochemical (IHC) staining on SurePath™ urine samples and biopsy were performed. Fascin staining was also performed on five negative control urine cytology and biopsy cases.

Results: Intense cytoplasmic staining was observed in malignant cells in all thirty-five urines (sensitivity =100%). Intense staining was not detected in benign urothelial cells in which only occasional faint, nonspecific cytoplasmic and nuclear staining was observed. All five negative control urines were also negative for Fascin staining (specificity =100%). All thirty-five (100%) corresponding biopsy cases with invasive urothelial carcinoma were positive for Fascin. Staining was generally intense and cytoplasmic. Weak to moderate staining was noted in the basal layer but was absent in the superficial urothelium of five negative control biopsy cases. In eleven cases (31%), Fascin immunoreactivity was observed in both the invasive and non-invasive components of the lesion examined. Lastly, in six cases (17%) Fascin highlighted isolated invasive tumor cells and was negative in the overlying non-invasive tumor component.

Conclusions: This study is the first to utilize Fascin IHC in cytology with biopsy correlation. Fascin staining can be successfully performed on SurePath™ cytology preparations. Fascin, while not a definitive marker for invasion, is overexpressed in urothelial carcinomas of the urinary bladder. Furthermore, intense Fascin staining in urine cytology specimens appears to be highly sensitive and specific for urothelial carcinoma and correlates with invasion on subsequent biopsy. Of particular interest is a subset of biopsy cases in which Fascin highlighted only isolated nests of invasive tumor cells; intense Fascin staining was noted in the concurrent urine cytology. Fascin staining on urine cytology is a useful adjunct to predict invasiveness in subsequent biopsies.

443 Differential Expression of PAX8, WT1, and Calretinin in Peritoneal Cytology: An Immunohistochemical (IHC) Study of Metastatic Ovarian Serous Carcinoma (OSC)

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Background: Metastases from primary ovarian neoplasms are common in peritoneal fluid. Microscopic evaluation has prognostic value as ovarian tumor staging incorporates the presence of malignant cells in peritoneal cytology. Reactive mesothelial cells can mimic OSC, and it is often difficult to distinguish the two on morphology alone. Calretinin by IHC has been recognized as a reliable marker for mesothelial cells, while, WT1 has proven useful in the diagnosis of OSC. This can be a diagnostic pitfall, as mesothelial cells can show immunoreactivity for WT1. Recently, PAX8 has been used as a marker in distinguishing ovarian from mammary carcinoma. To our knowledge

no studies using PAX8 have been performed on peritoneal cytology specimens, and its expression in metastatic OSC has not been studied.
Design: IHC for PAX8, WT1, and Calretinin were performed on paraffin-embedded cell block sections from forty-one metastatic OSC peritoneal cytology cases and ten negative controls. Sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) for the cytology results were calculated using histological diagnosis as the gold standard. Differential expression of PAX8, WT1, and Calretinin was also evaluated in background mesothelial cells.
Results:

Marker expression in OSC and Negative controls.

	PAX8	WT1	Calretinin
OSC cases	37/41 (90%)	38/41 (93%)	0/41 (0%)
Negative controls	0/10 (0%)	10/10 (100%)	10/10 (100%)

Sensitivity and specificity data of markers used

	Sensitivity	Specificity	PPV	NPV
PAX8	90%	100%	100%	71%
WT1	92%	0%	79%	0%

In benign and positive peritoneal cytology fluids both WT1 and Calretinin staining were observed in background mesothelial cells, consistently with variable intensity. PAX8 expression was not observed in mesothelial cells, and Calretinin expression was not observed in ovarian carcinomas.
Conclusions: PAX8 is a sensitive and specific marker for the detection of metastatic OSC and Calretinin is useful for identifying mesothelial cells. While PAX8 and WT1 demonstrate comparable sensitivity (90% and 93% respectively) in diagnosing metastatic OSC, PAX8 has superior specificity as staining is not observed in mesothelial cells. This suggests that the profile of PAX8-positive, Calretinin-negative is highly specific and sensitive for detecting metastatic OSC and can be useful in distinguishing it from mesothelial cells.

444 The ER Antibody SP1 May Not Distinguish Breast from Pulmonary Adenocarcinomas in Malignant Pleural Effusions

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Background: The most common primary sites for adenocarcinomas in malignant pleural effusions include lung and breast. The distinction is clinically important for diagnosis as well as therapeutic management. Morphologic overlap between adenocarcinomas of breast and pulmonary origin often require the use of cell or lineage-specific immunocytochemical markers for this differential diagnosis. TTF-1 is expressed by 75% of primary pulmonary adenocarcinomas and has not been described in carcinomas of breast origin. Estrogen receptor (ER) is expressed by 75% of breast adenocarcinomas but expression in pulmonary adenocarcinomas has been variable and antibody dependent. We investigated the immunocytochemical expression of ER in proven primary lung adenocarcinomas using 1D5, a mouse monoclonal antibody and SP1, a recently described rabbit monoclonal ER antibody.
Design: Forty-two pleural effusions diagnostic for metastatic pulmonary adenocarcinomas (14 women and 28 men) confirmed by clinical presentation and positive immunocytochemistry for TTF-1 were included in this study. Immunocytochemistry for ER using 1D5 and SP1 was performed on alcohol-fixed, previously Papanicolaou-stained slides, using the L-SAB detection system. Any nuclear reactivity for ER was considered a positive result.
Results: A positive nuclear reaction for ER using SP1 was detected in 10 (23.8%) effusion specimens diagnostic for metastatic pulmonary adenocarcinoma. None of the cases were positive for 1D5.
Conclusions: The rabbit monoclonal ER antibody SP1 may yield positive results in pulmonary adenocarcinomas. Caution should therefore be exercised in the use of this antibody alone in distinguishing metastatic breast from pulmonary adenocarcinomas in malignant pleural effusions.

445 Reflex HPV Testing in Vaginal Smears

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Background: High-risk human papillomavirus (hrHPV) testing for triage of Papanicolaou smears with an interpretation of atypical squamous cells of undetermined significance (ASCUS) is well established. Current management recommendations for ASCUS are: hrHPV testing, repeat cervical cytology, or colposcopy; although hrHPV testing is preferred. These recommendations are for cervical specimens; the original studies excluded women with a prior hysterectomy. In clinical practice, reflex hrHPV testing is performed on vaginal smears with ASCUS. Few studies have assessed hrHPV testing in vaginal smears. The purpose of this study is to see if hrHPV triage in vaginal smears is informative.
Design: A retrospective review was performed on vaginal smears with an interpretation of ASCUS and reflex hrHPV testing collected from January 2006 to December 2007 and correlated with follow-up cytology and histology.
Results: An interpretation of ASCUS was rendered on 96 of 2949 vaginal smears. The ASCUS rate was 3.2% compared to an overall ASCUS rate of 9.8% at the same institution in 2007. HPV reflex testing was performed on 56/96. There were 47 hrHPV negative cases and 9 hrHPV positive cases for an overall ASCUS hrHPV positive rate of 16%. Follow-up vaginal smears were available in 44 patients and a follow-up biopsy was available in one patient.

	Vaginal Smear Follow-up				
	NEG	ASCUS	SIL	TOTAL	AGE
ASCUS HPV -	25	5	7	37	57 (21-76)
ASCUS HPV +	4	1	2	7	50 (39-63)

A squamous intraepithelial lesion (SIL) was identified in nine patients; two from the hrHPV positive group and seven from the hrHPV negative group. All of the SIL was interpreted as low-grade, except for one patient in the hrHPV positive group who had a high-grade squamous intraepithelial lesion. This patient had a hysterectomy for severe cervical dysplasia and had recurrent vulvar squamous dysplasia. There was no significant difference in the diagnosis of SIL between the hrHPV positive group and the hrHPV negative group (p=0.67).
Conclusions: Although this study had a small sample size, the observed 16% hrHPV positivity rate is well below the reported rate in cervical specimens of approximately 40%. The low prevalence of hrHVP in vaginal specimens may explain the low ASCUS rate. There was no significant difference in follow-up between hrHPV positive and hrHPV negative ASCUS vaginal smears. These results suggest that hrHPV triage does not provide additional information. Perhaps a different testing algorithm with increased follow-up may be considered in place of hrHPV triage in vaginal smears with ASCUS.

446 FN1, GALE, MET and QPCT mRNA Overexpression in Papillary Thyroid Carcinoma Using Routine FNAB Samples and Frozen Tissue

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Background: Thyroid nodules are frequent in clinical practice and fine needle aspiration biopsy (FNAB) is widely used for its evaluation. Only 5% of the cases are diagnosed as malignant, being papillary carcinoma (PC) the most common neoplasia, and approximately 20% are classified as indeterminate or suspicious for malignancy. In a prior study, we were able to apply qRT-PCR method in a series of routinely performed FNAB of thyroid nodules using individual, residual samples. In the present study, we used this series to evaluate the expression of 6 genes related to PC, comparing it with a set of frozen thyroid tissue from surgical specimens.
Design: A total of 70 thyroid samples were evaluated for the expression of *MPPED2*, *H/HBA2*, *MET*, *FN1*, *GALE* and *QPCT* genes, including 24 cases of frozen thyroid tissue (12 nodular hyperplasia and 12 papillary carcinoma) and 46 consecutive thyroid FNAB samples previously analyzed (3 positive, 3 indeterminate and 32 negative for malignancy, 7 most probably benign and 1 suggestive of benign follicular lesion). The ΔΔCT method was used to calculate the relative expression of the 6 target genes and fold change in expression was calculated as $2^{-\Delta\Delta CT}$.
Results: As seen on Figure 1A, *FN1*, *GALE*, *MET*, and *QPCT* mRNA expression were found to be significantly different in benign and malignant frozen thyroid samples (p = 0.0051, p = 0.0010, p = 0.0074 and p = 0.0102, respectively); FNA samples showed a similar pattern of overexpression (Figure 1B), although only 3 malignant aspirates were analyzed. *H/HBA2* and *MPPED2* results varied (p = 0.6442 and p = 0.549, respectively).

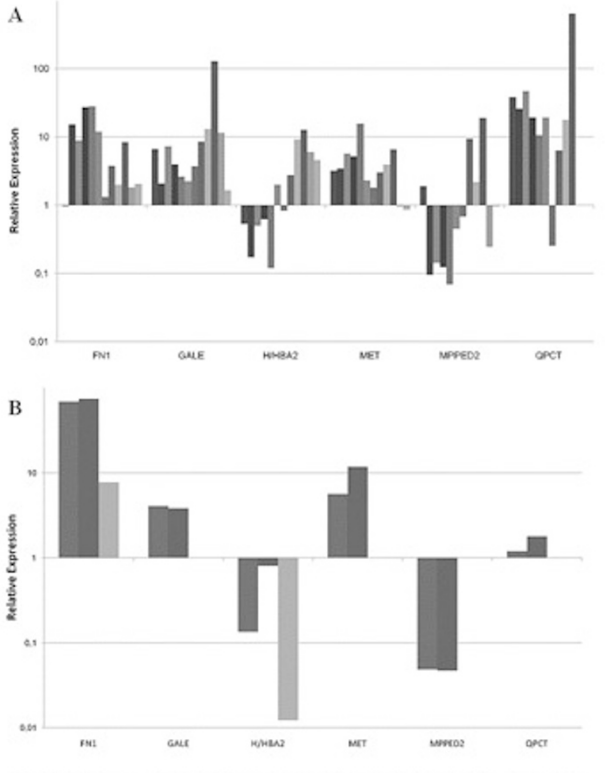

Figure 1. Expression pattern of the six target genes in frozen thyroid tissue (A) and FNAB samples (B).

Conclusions: *FN1*, *GALE*, *MET* and *QPCT* are significantly overexpressed in malignant thyroid tumors, representing possible molecular markers to be used for pre-operative diagnosis. RT-PCR analysis using individual, residual samples of thyroid nodules aspirates can be employed to access gene expression, adding elements for final cytological diagnosis of indeterminate or suspicious cases, without altering the routine procedure.

447 Inclusion of the Uniform Tetraploid Cells Reduces the Specificity of the Urine FISH Assay

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Background: There are approximately 50,000 new cases and 10,000 deaths from urinary bladder urothelial neoplasia each year in the United States. Cytology alone has high specificity but low sensitivity for bladder cancer detection. To increase the sensitivity, a FISH assay has been utilized to detect chromosomal abnormalities. Existing literature shows a wide variability in sensitivity and specificity of the FISH assay as performed in different centers. In this study we have identified a feature that deteriorates the specificity of the test.

Design: Among 215 cases who had urine FISH (UroVysion) test at the VAMC, 45 had associated histopathology. According to the FDA criteria, for a positive diagnosis of urothelial neoplasia, a minimum of 4 cells with hyperploidy of at least 2 of the 4 chromosomes or a minimum of 12 cells with homozygous loss of P16 gene are required when a minimum of 25 cells are examined. The four probes are for CEP (chromosomes 3, 7, 17) and P16 on chromosome 9. In this study, a cell with four signals for all four chromosomes was classified as a uniform tetraploid cell (UTC); a presumed reparative cell. Positive and negative diagnoses by the FISH were made before and after subtracting the UTCs.

Results: Before subtracting the UTCs, 22 cases were positive and 13 negative with 9 false positive and 1 false negative findings as compared with the histopathologic diagnoses. In this category, the sensitivity and specificity were 95.7% and 59.1% respectively. After subtraction of the UTCs, 21 cases became positive and 20 negative with a significant reduction in the false positive results. By excluding the UTCs, the assay performance significantly improved for all the four parameters.

Summary of the urine FISH assay before (+) and after (-) exclusion of the UTCs

	True Positive	True negative	False Positive	False Negative	Sensitivity	Specificity	PPV - NPV
+Tetraploid	22	13	9	1	95.7%	59.1%	71.0%-92.9%
-Tetraploid	21	20	2	2	91.3%	90.9%	91.3%-90.9%

Conclusions: Although this FISH assay has offered a much higher sensitivity than routine cytopathological examination, it has suffered from its variably lower specificity as used in different centers and laboratories. If, however, the UTCs are excluded as positive cells from the FDA criteria, this FISH assay would offer a more solid performance than its usage with the current criteria.

448 Myoepithelial Carcinoma of the Salivary Glands: Cytomorphologic Characteristics and Differential Diagnosis

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Background: Myoepithelial carcinomas (MC) are extremely uncommon accounting for < 0.1% of all primary salivary gland neoplasms. Cytomorphologic descriptions of MC are rare, mostly limited by case reports. The current study from a single large institution elaborates on morphologic criteria and differential diagnoses of MCs.

Design: A retrospective search of the cytopathology archives from a large tertiary care center for a 19-year period (1989-2008) revealed ten cases of MCs in eight patients (primary-9, metastatic-1). Material was obtained by fine needle aspiration (FNA) performed with or without radiologic guidance. Smears were stained with Diff Quik and Papanicolaou stains. Clinical outcome and histopathologic follow-up was additionally reviewed and correlated.

Results: There were two males and six females (M: F, 1:3), ranging in age from 34-87 years (mean age 63.6 yrs). The anatomic locations were; parotid (5), submandibular (2), parapharyngeal (2), lung (1). The most common clinical presentation was an incidental mass or facial pain. The tumor size ranged from 1.5-3.5 cm (mean size 2 cm). The initial FNA diagnoses were: salivary gland neoplasm, NOS (4), epithelial neoplasm, NOS (3), pleomorphic adenoma [PA] (3). The histologic diagnoses were; MC (8) and high-grade MC (2). Cytomorphologic characteristics were; hypercellular smears, predominantly discohesive single cells, syncytial or papillary-like fragments with perivascular nesting, rare/focal metachromatic stroma, plasmacytoid to epithelioid cells with cytoplasmic tails, oval to spindled/fusiform nuclei, cells with clear/vacuolated cytoplasm and abundant naked nuclei. A key feature was cellular monotony with only slight anisonucleosis. Cases with high-grade MC follow-up disclosed mitoses and karryorhexis. Immunostaining was done in seven cases using S100 protein, epithelial and smooth muscle markers and displayed expected results. Clinical outcome was available in all cases-alive without disease (8).

Conclusions: FNA interpretation of MC is extremely difficult due to its rarity and highly variegated cytomorphologic appearance leading to a more generic cytologic diagnosis of "salivary gland neoplasm, NOS". Cellular monotony with mostly discohesive cells or papillary-like phenotype in a stroma-poor lesion should raise the possibility of an MC. Immunostaining can be helpful in selected cases. Differential diagnosis includes PA, myopeithelioma, and metastatic melanoma.

449 Success of EUS-FNAB and Flow Cytometry in the Diagnosis of Deep-Seated Lymphoproliferative Processes

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Background: Endoscopic ultrasound-guided fine-needle aspiration biopsy (EUS-FNAB) has allowed the sampling of deep tissues, including deep-seated lymph nodes. EUS-FNAB alone is an effective tool in the diagnosis of metastatic epithelial neoplasms in deep-seated lymph nodes. However, the literature regarding the use of EUS-FNAB in the lymphoproliferative process and the yield of flow cytometry specimens via EUS-FNAB is sparse. The aim of this study is to explore the utility of EUS-FNAB in the diagnosis of both deep-seated lymphoma and reactive conditions with an emphasis on the number of passes required to obtain adequate cellularity for flow cytometry confirmation of the diagnosis.

Design: A retrospective search was conducted at our tertiary care center on all EUS-FNAB specimens obtained from January 2006-June 2009. Out of 2140 specimens identified, 706 were aspirates from deep-seated lymph nodes. Ninety-one lymph node aspirates submitted for flow cytometry were reviewed, yielding diagnoses of lymphoma, benign lymph node, reactive lymph node, or metastatic lesion to a lymph node. Flow cytometry data, including the number of passes and of viable cells, were also evaluated.

Results: After two to three passes, the total yield of viable cells per case diagnosed by flow cytometry ranged from 4.0×10^{-3} million to 1.33×10^{1} million (mean 3.14 million). Nine of the cases submitted for flow cytometry yielded a quantity of cells insufficient for diagnosis, including three reactive lymph nodes, one metastatic lesion to a lymph node and five benign lymph nodes. Thirty-one specimens from 29 patients (18 males and 11 females aged 39 to 86 years) yielded the diagnosis of lymphoma. There was only one case in which flow cytometry did not confirm malignancy. The diagnosis of lymphoma was supported by surgical biopsy or excision in ten of the cases and by immunohistochemical staining in eight cases. Flow cytometry combined with EUS-FNAB and/or surgical follow up supported the diagnosis of lymphoma in all 31 specimens. In the non-lymphoma cases, 18 specimens showed a reactive process, 8 showed a metastatic lesion, and 34 showed a benign process.

Conclusions: Combined use of EUS-FNAB and flow cytometry can be a powerful tool for the diagnosis of a deep-seated lymphoproliferative process. At least 2 to 3 passes are required for flow cytometry specimens to obtain good viability. Molecular studies on EUS-FNAB specimens will aid in the diagnosis and detection of minimal residual disease.

450 Raman Spectroscopy: A Novel Tool for Cervical Cancer Screening

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Background: Cervical cancer is the second most common cancer in women worldwide. The Papanicolaou test is used to detect cancer and pre-cancer in the general female population but it is widely acknowledged that sensitivity values are low. The aim of this study was to investigate the potential of Raman spectroscopy to detect biochemical changes in cervical smear samples. The ability of Raman spectroscopy to classify different cell types, discriminate between nuclear and cytoplasmic regions of squamous epithelial cells and between normal and abnormal cervical cells was investigated. Four cervical cancer cell lines were also investigated to determine if cell lines with different HPV copy number could be discriminated.

Design: Cervical smear samples were obtained from CWIUH. C33A (HPV negative), HeLa (HPV-18 positive, 20-50 copies per cell), SiHa (HPV-16 positive, 1-2 copies per cell) and CaSki (HPV-16 positive, 60-600 copies per cell) cells were cultured on glass slides. Raman measurements were carried out using a Horiba JY Labram HR800 Raman microscope. After Raman measurements, cells were stained with Papanicolaou stain, coverslipped and submitted for cytological examination. Subsequently, the Raman spectra were analysed using Principal Component Analysis (PCA), a multivariate statistical technique.

Results: The Raman spectra showed differences in the glycogen, protein and nucleic acid levels of superficial epithelial cells, navicular cells and polymorphs, as well as between the nucleus and cytoplasm of superficial squamous epithelial cells. In the abnormal cervical cells, a significant increase in nuclear activity was observed, as indicated by an increase in the intensity of peaks assigned to nucleic acids. The cervical cell lines showed distinct differences in their Raman spectra. Principal Component Analysis (PCA) successfully discriminated different classes of cervical cells, cell regions, and normal from abnormal cells. In addition, the cervical cell lines were discriminated by PCA based on their HPV copy number.

Conclusions: The results show clearly that Raman spectroscopy can be used for identification and discrimination of normal and abnormal cervical cells. Moreover, HPV positive and negative cell lines could be discriminated based on their biochemical fingerprint. Raman spectroscopy thus shows enormous clinical potential for cervical cancer screening.

451 Fixative Type Significantly Affects S100P Immunoreactivity in Fine Needle Aspiration Biopsies of Pancreatic Ductal Neoplasms

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Background: Evaluation of pancreatic lesions is frequently based on limited material obtained by fine needle aspiration biopsy (FNAB). S100P has shown promise as an ancillary marker of neoplasia. In this study, we further evaluate the utility of S100P in distinguishing benign epithelium from ductal neoplasms in FNAB and specifically evaluate the effect of fixative solution.

Design: A tissue microarray (TMA) study set of 321 resections consisted of 77 ductal adenocarcinoma (PDAC), 73 benign pancreas, 50 intraductal papillary mucinous neoplasm (IPMN), 20 mucinous cystic neoplasm (MCN), and 36 PanIN, as well as 61 normal small intestine and stomach. A test set of paraffin-embedded cell block material from 57 FNAB included 42 PDAC (16 confirmed on resection), 4 atypical cases (confirmed as PDAC on resection), 2 mucinous neoplasms (confirmed on resection), and 9 benign ductal epithelium. S100P immunohistochemistry (BD Biosciences, clone 16) was performed and scored as negative (no staining), 1+ (1-25%), 2+ (25-75%), and 3+ (>75%).

Results: In the study set, 70/73 PDAC exhibited 3+ S100P. 1/73 benign pancreas exhibited 1+ S100P. IPMN (50/50), MCN (18/20), and PanIN (36/36) exhibited 3+ S100P reactivity. Compared with benign pancreas, S100P was 99% sensitive and 99% specific for ductal neoplasms. 19/20 normal gastric mucosa demonstrated 3+ S100P while normal small bowel was S100P negative (0/27). S100P labeled 29/42 (69%) PDAC, 4/4 of atypical cases, and 2/2 mucinous neoplasms (Table 1). Compared with

benign pancreas, S100P was 72% sensitive and 100% specific for ductal neoplasms. Cytolyte-fixed material from pancreatic neoplasms was less often S100P-positive (20/32) compared with formalin-fixed tissue (14/15) (p=0.03). S100P was positive in 8/19 gastrointestinal mucosa from EUS-FNAB.

Table 1. S100P Immunohistochemistry in FNAB Test Set

Cytologic Findings	No. of Cases	S100P Negative	S100P 1+	S100P 2+	S100P 3+	Cytolyte Fixative (Pos/Total)	Formalin Fixative (Pos/Total)
Adenocarcinoma	42	13	5	7	17	18/30	11/12
Atypical	4	0	0	2	2	1/1	3/3
Mucinous Neoplasm	2	0	1	0	1	1/1	1/1
Benign Ductal Cells	9	9	0	0	0	0/3	0/6
Gastrointestinal Mucosa	19	11	1	3	4	4/13	4/6

Conclusions: S100P is a sensitive marker of ductal neoplasia in the pancreas, but cannot distinguish between PDAC and its precursors. S100P cannot distinguish between mucinous neoplasia and gastric mucosal sampling in EUS-FNAB. For optimal results, specimens should be fixed in formalin and not cytolyte.

452 Fine Needle Aspiration (FNA) of 486 Human Immunodeficiency Virus (HIV)-Related Lesions of the Major Salivary Glands

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Background: Salivary gland disease is an important manifestation of HIV infection. There are limited studies investigating the cytopathology of HIV-related salivary gland disease. The aim of this study was to evaluate the findings in a series of salivary gland FNA in South African HIV+ patients.

Design: A retrospective review at the National Health Laboratory Service in Johannesburg, South Africa was performed of 3501 confirmed HIV+ patients who had an FNA of various body sites. FNA of only major salivary glands were further evaluated for patient age and gender, lesion (laterality, clinical signs), antiretroviral therapy use, specimen adequacy, and cytologic diagnosis.

Results: There were 486 (14%) procedures including 253 (52%) parotid and 233 (48%) submandibular gland FNAs. Patients were of average age 34 years (range, 3-63) with a female:male ratio of 1:0.6. There were 43 (9%) inadequate FNAs and 22 (5%) contained only normal gland constituents. Most diagnoses were due to 123 (25%) lymphoepithelial lesions/cysts and 114 (23%) cases of reactive lymphadenopathy. Lymphoepithelial lesions/cysts occurred in patients ranging from 6-63 years of age, 7% of whom had antiretrovirals, measured 2 to 8.5cm in greatest diameter, involved the parotid (n=94) and submandibular glands (n=81), and were bilateral in only 7% of patients. Cases also included 81 (17%) diagnoses with tuberculosis and 58 (12%) abscesses, 2 found to have associated mycobacterium infection. Neoplasms accounted for 17 (4%) of diagnoses, including pleomorphic adenoma (n=9), lymphoma (n=7) and squamous cell carcinoma (n=1). Those with pleomorphic adenomas were of average age 38 years (range, 32-44). There were 13 (3%) atypical cases (possible neoplasms) and 13 miscellaneous diagnoses (10 non-specific sialadenitis, 3 epidermal inclusion cysts).

Conclusions: These data indicate that FNA is useful to evaluate salivary gland lesions in a HIV+ population and may reduce their need for surgery, an important consideration in an underfunded public health care system. HIV-related salivary gland disease in South Africa is mainly due to benign conditions including lymphoepithelial lesions/cysts, reactive lymphadenopathy, and coinfection like tuberculosis. Neoplasms account for only a minor component of diagnoses in this HIV+ population.

453 TTF-1, Napsin A, and Surfactant A to Identify Lung Carcinoma in Malignant Pleural Effusions – Are More Stains Better Than One?

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Background: Lung carcinoma (LC) is the leading cause of cancer deaths in the U.S. The lungs are also common sites of metastases from extrapulmonary primaries (EXP). Optimal clinical management often requires clarification of the origin of tumor cells in malignant pleural effusions (PE). TTF-1 (TTF1), napsin A (napsin), and surfactant A (surfactant) immunostains are used to support a diagnosis of LC. TTF1 is frequently the initial stain ordered given that thyroid carcinoma rarely involves PE. This study evaluates napsin and surfactant expression in a series of PE with TTF1 positive (TTF1+) and TTF1 negative (TTF1-) LC and EXP.

Design: Serial sections of formalin fixed, paraffin embedded cell blocks from 92 PE with histologically and/or clinically confirmed LC (N=58) and EXP (N=34) were immunostained for TTF1, napsin, and surfactant. LC PE included 30 TTF1+ and 28 TTF1- cases, and EXP PE included 30 carcinomas (10 breast, 5 mullerian, 3 pancreas, 3 colon, and 1-2 each of stomach, prostate, cervix, larynx, kidney, bile duct) and 4 mesotheliomas. Staining intensity (weak (1+), moderate (2+), strong (3+)) and percentage of tumor cells stained were recorded for each case. Staining was considered positive when intensity was 1+ in ≥10% of tumor cells or ≥2+ in any number of tumor cells. Results were correlated with final diagnoses.

Results: Among the TTF1- LC PE, 8 (29%) and 12 (43%) cases were positive for napsin and surfactant, respectively. Both stains were positive in 7 of these cases. Among the TTF1+ LC PE, all 3 stains were positive in 20 (67%) cases, with napsin positive in 24 (80%) and surfactant positive in 23 (77%) cases. More than 75% of tumor cells were positive in 80% (24/30) of all TTF1+, 84% (27/32) of all napsin positive, and 35% (14/40) of all surfactant positive malignant PE. Among the EXP PE, TTF1, napsin and surfactant were negative in 34 (100%), 34 (100%), and 29 (85%) cases, respectively. Five (15%) of the EXP PE (2 colon, 2 breast, 1 cervix) were positive for surfactant, with staining observed in 5%-60% of tumor cells.

Conclusions: TTF1 and napsin exhibited similar sensitivities and specificities indicating that they are comparable as initial stains for LC in PE. Both napsin and surfactant identified several of the TTF1- LC PE; however, the 100% specificity of napsin rendered it more reliable than surfactant as an initial and 2nd tier stain for LC. When both TTF1 and napsin are negative, a positive stain for surfactant should only be interpreted in the context of a broader panel of immunostains.

454 Positive Peritoneal Washes in Robotic Assisted Laparoscopic Hysterectomy for Endometrial Carcinoma

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Background: Laparoscopic hysterectomy is reported to have an artifact of vascular pseudoinvasion, especially seen in low risk endometrial carcinoma cases. This has been attributed to the use of the uterine balloon manipulator technique during laparascopy. This is seen as endometrial glands and stroma in large caliber vascular spaces. We studied our cases of endometrial carcinoma to determine if a similar artifactual dissemination was noted with pelvic washings in patients with Robotic Assisted Laparoscopic Hysterectomy.

Design: A review of our pathology data base was conducted to find all cases of endometrial carcinoma with pelvic washings during a 22 month period (since the use of robotic surgery for endometrial carcinoma at our institution). The clinicopathologic parameters of all cases of endometrial carcinoma with positive washings were compared, with special attention to the surgical technique used.

Results: 95 cases of EMCA were staged over a 22 month period at our institution. Of these 95 cases, 45 had Robotic Assisted Laparascopic Hysterectomy (RALH). 24 cases were high grade carcinoma (including 20 cases of serous, clear cell and malignant mixed mullerian type- so called type 2 endometrial carcinoma), only 8 of which were staged using RALH. Washings were positive in 15 cases, of which 8 were in RALH cases. The RALH cases consisted of 5 low endometrioid carcinomas and 3 serous carcinomas. Of the non-robotic cases 4 were serous, 2 endometrioid and 2 were MMMT. There were no appreciable differences between the patients with RALH and positive washings, and non robotic surgery and positive washings. The number of cases with low grade carcinoma with positive washings was slightly higher in the RALH group (5/37 (13.5%) versus 4/31 (12.1%)) but did not achieve statistical significance.

Conclusions: The factors that cause artifactual tumor dissemination into vascular channels during laparoscopic assisted hysterectomy, do not appear to result in tumor contamination of the peritoneal washings. More detailed studies, with a larger cohort of cases, are needed to verify this result.

455 Molecular-Cytologic Correlation of Pancreatic Cystic Lesions (PCL): Our Experience

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Background: Recently molecular analysis (MDX) of pancreatic cyst fluid has been included in the armamentarium of ancillary tests to assess the multidisciplinary preoperative diagnosis of PCL. Few studies show correlation among these diagnostic tools. The purpose of the study is to present our experience.

Design: MDX PathFinderTG® (RedPath Integrated Pathology, Pittsburgh, PA) reports corresponding to PCLs received in the past 11 months were retrospectively reviewed. Comparison with cytologic diagnosis (CDX), CEA fluid level (> 192 ng/ml P positive, <192 ng/ml N negative, NA not available) and surgical diagnosis (SDX) was performed. Cases were divided in 4 categories including, benign non mucinous neoplasms (BNM), mucinous neoplasms (MN), malignant and non-diagnostic (ND).

Results: A total of 27 PCL including 17 females and 10 males, within 32 to 83 years of age were analyzed. PCLs were located in the head (12), body (9) and tail (5). 26 PCLs had concurrent CDX and only 4 cases had SDX (table1).

1. PCL correlation

CEA	**MDX**	**CDX**	**SDX**
P	MN	MN	IPMN
NA	MN	BNM	MCA
N	MN	MN	IPMN
N	BNM	SSPT	SPPT

SSPT solid pseudopapillary tumor, MCA mucinous cystadenoma, IPMN intraductal papillary mucinous neoplasm

Among those, 2 correlated and the other 2 were incorrectly classified, one by each technique. CEA fluid level was available in 3 cases, 2 were concordant and 1 was not. MDX detected 14 BNM, 10 MN and 2 ND cases (table 2).

2. Correlation: MDX Vs. CDX

		CDX		
CATEGORIES	MDX	C	NC	ND
BNM	14	9	2	3
MN	10	3	1	6
ND	2			

C corelate, NC non correlate

No malignant diagnosis was rendered. CDX & MDX correlation was better for BNM (64%) than MN (30%). MN had a more ND cases on CDX . All the ND-CDX were reviewed. Four cases had scant slightlly atypical epithelium, favoring MN, 2 of them with positive concordant CEA. 2 cases had macrophages, compatible with BNM and 3 cases were virtually acellular (true ND). 2 cases had mucinous epithelium interpreted as gastrointestinal (GI) origin and 1 had acute inflammation.

Conclusions: Longer follow up is necessary to determine value of MDX. Greater proportion of MN is detected by MDX Vs CDX. Cytology is better at diagnosing BNM than MN, careful and descriptive examination may reduce the rate of ND CDX, especially in MN. Cytological interpretation is limited when evaluating benign mucinous epithelium: neoplastic Vs GI contaminants. Molecular and cytologic criteria need to be strictly defined to grant a better assessment of PCLs in future studies.

456 ThinPrep® (TP) Morphology of Amebic Cysts in Anal Cytology Samples: Morphologic Features and Comparison to Conventional Smears

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Background: Although the cytomorphology of amoebae is known from conventional smears (CV), their features in TP have not been previously described. In this case series, we describe the morphological features of various amebic cysts in anal TP (ATP).

Design: Files from 01-2006 through 09-2009 were reviewed to identify all anal/rectal TP which were positive for amoebae. Cytomorphological characteristics, including background, preservation, size (S), cytoplasm (C), number of nuclei (N), chromatoid bodies (CB) and capsule were evaluated and compared.

Results: A total 1527 ATP specimens, all referred from the infectious disease clinic as high risk patients for anal squamous intraepithelial lesions, were found. 0.7% (n=11 cases) were positive for parasites (14 organisms). 13 amoebic cysts were identified, including *Entamoeba histolytica* (EH), *Entamoeba coli* (EC), *Iodamoeba bütschlii* (IB), *Endolimax nana* (EN), 1 case had a protozoan cyst *Blastocystis hominis* (BH). IB was the most common identified parasite (50%). 3 cases had 2 organims: EC and IB (2) and EH and BH (1). All cases revealed: clean background, no obscuring artifacts, good cellular detail, double contour membrane, crisp and easily visualized internal structures. All parasites were separated from surrounding cellular material,and were easily visible; except for one case that showed degenerative changes. All parasites were in the cyst stage. Morphologic comparisons of the cysts with those described in CV are displayed in the table.

Comparison of amebic cysts, CV Vs ATP

CYSTS	CHARACTERISTICS	CV	ATP
EH (3)	S	5-20	9 (8-10)
	CYT	diffuse glycogen, mahogany brown	soft, light blue
	CB	large bars or thick rods	thick rod, dark blue
	NC	1-4	4
EC (3)	S	17 (10-33)	17 (13-19)
	CYT	large mass, ill defined dark brown	dense, dusky blue
	CB	sometimes, splinter like	-
	NC	1-8	8
IB (6)	S	10 (5-18)	10 (8-10)
	CYT	large mass, compact, dark brown, vacuole	finely granular, dark blue,translucent vacuole
	CB	-	-
	N	1-2	1 (dark, piknotic)
EN (1)	S	9 (5-14)	7
	CYT	absent, ill-defined brown	granular, blue
	CB	small, spherical or elongated granules	red, small, round granules
	N	1-4	1-2

Conclusions: Amebic parasites were easily found and recognized in ATP Compared to the CV, size of the organism was smaller and the color of the cytoplasm appears to be blue, rather than brown in ATP TP offers: clean background, excellent preservation and a smaller slide surface to screen.

457 Liquid-Based Cytology Improves the Identification of Malignant Tumors in Thyroid Fine-Needle Biopsies

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Background: Fine-needle aspiration cytology (FNAC) is the most important tool for correctly diagnosing thyroid malignant tumors. The introduction of liquid-based cytology (LBC) has allowed the standardization of the technical processing of the aspirations and the better identification of the malignant features of the follicular cells. The aim of this study is to assess the efficacy of the LBC in identifying malignant neoplasms in suspicious lesions sampled by FNA.

Design: In 2008 2,622 thyroid biopsies have been carried out in the Division of Pathology of the Catholic University of Rome under sonographic guidance.All biopsies were processed according to the Thin Prep 2000 method (Hologic,Marlborough,MA). Among them 333 (12.7%) were classified as follicular neoplasm (Thy 3 according to the British Thyroid Association, BTA) and 85 (3.2%) as suspicious for malignant tumor (Thy 4 according to the BTA). The overall rate of suspicious lesions accounted for 15.9%. Among these patients 199 (47.6%) underwent surgery: 136 diagnosed as Thy 3 (40.4%) and 63 diagnosed as Thy 4 (74.1%).

Results: The overall rate of malignant occurrence in suspicious thyroid lesions was 19.6%. In Thy 3 the malignant neoplasms represented 26.4% (36 out of 136 patients addressed to surgery) whereas the Thy 4 malignancy rate was 73% (46 out of 63 operated patients). The comparison with previous periods (2004 with LBC only, 2001-2002 with LBC plus conventional smears, CS, and 1995 with only CS) shows a statistically significant increase of both malignancy rates. In fact the Thy 3 category malignancy occurrence was 14.4% in 2004, 11.5% in 2001-2002 and 2.1% in 1995 whereas the same rate of malignancy in Thy 4 lesions was 53.4% in 2004, 77.5% in 2001-2002 and 46.1% in 1995.The PPV and NPV were respectively 73 and 73.5%

Conclusions: The adoption of the LBC cytology may help in identifying more correctly than CS the cases showing malignant features on thyroid fine-needle biopsies. Rossi ED et al.Diagnostic Efficacy of Conventional as Compared to Liquid-Based Cytology in Thyroid Lesions. Acta Cytol.(in press)

458 Improved Predictive Value Provided by ProExC™ Staining of HSIL Pap Smears Supports Leap to LEEP Clinical Management

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Background: Management guidelines now allow for a "leap to LEEP" in non-adolescent women with a cervical pap smear diagnosis of high-grade dysplasia (HSIL). This change was due to an improved positive predictive value (PPV) of HSIL (70-80) provided by liquid-based paps sufficient to bypass inefficient colposcopy. In our experience, however, the consequence has been a reduction in the frequency of definitive HSIL diagnoses. Our clinicians are also hesitant to LEEP because of significant false positive rates. HPV testing is not useful because the PPV is no better than a HSIL diagnosis. Our objective was to test whether a marker of neoplastic transformation, such as ProExC™, provides an improved PPV to better support "leap to LEEP."

Design: SurePath™ cervical pap smears diagnosed as HSIL (n=118) at either OHSU or Kaiser Northwest were immunostained for ProExC (BD Tripath) using a Ventana Benchmark XT. A minimum of 5000 epithelial cells per slide were required to be adequate for immunostaining and cases were scored as positive or negative by a cytopathologist (TM) and resident (CR). Each specimen was tested for high-risk HPV by hybrid capture (Digene™) and all followup cervical LEEP biopsies were immunostained for ProExC to generate a "Clinical Consensus Diagnosis" as the gold standard surgical outcome. Outcomes were classified as either biopsy-proven high grade dysplasia (CIN 2+), or a minimum of two years negative followup (including CIN 1). Predictive values were calculated and associations determined by Chi-square analysis.

Results: We observed excellent agreement between pathologists' scoring of the immunostained pap smears and biopsies (kappa statistic 0.61, 0.78, respectively). Discordant scores occurred in paps with fewer than 10 positive cells (52% discordance, compared to 3-5% in negative or abundantly positive cases). The prevalence of CIN 2+ in this HSIL cohort was 81%. Chi-square analysis showed a significant association between positive ProExC staining and CIN2+ followup ($p<.0001$). Positive staining significantly improved the PPV (95%) and positive likelihood ratio (4.5 [CI:2-9]). The prevalence of high-risk HPV was 96% and the PPV of a positive test (81%) was no better than HSIL cytology.

Conclusions: Immunostaining cervical pap smears diagnosed as HSIL for ProExC significantly improves the PPV for CIN2+ surgical outcome, which may strengthen clinical confidence when leaping to LEEP in non-adolescent women.

459 Utility of Telecytopathology for Rapid Preliminary Diagnosis and Adequacy Assessment of Fine Needle Aspiration Biopsies

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Background: Telepathology, the practice of pathology at a distance, has been successfully tried for over two decades in various areas of pathology from medical education to diagnostic pathology, including consultation and frozen section diagnosis. However, the use of telepathology by cytopathologists has been limited to date. We present our institution's experience of using telecytology in rendering a preliminary diagnosis and adequacy assessment of Fine Needle Aspiration (FNA) procedures.

Design: A total of 39 cases were included in the study including a double blinded retrospective study of 15 cases and 24 cases performed prospectively. A telepathology system consisting of Olympus CX41 microscope and digital camera with NetCam software was used to transmit the images. The microscope was operated by different cytotechnologists and fellows who communicated with the viewing cytopathologists on a speaker phone. Comparison was made between preliminary diagnosis rendered by telepathology and the final diagnosis. Reasons for discrepancies were analyzed.

Results: The overall concordance rate between the preliminary and final diagnoses was 92.3%. The specimens were obtained from lymph nodes (14), thyroid (11), pancreas (7), bone (3), lungs (2) and head and neck (2). The final diagnoses were categorized as non-diagnostic in 3(7.7%), benign in 19(48.7%) and malignant in 17(43.6%) cases. Of the 15 cases selected for the retrospective study, 13(86.6%) showed concordance between the preliminary and final diagnoses. The two cases in which the diagnoses did not tally were both called 'atypical' initially. The final diagnosis was 'non small cell carcinoma' in one and 'benign ductal cells' in the other. In the first case, the diagnostic cells were not focused by the microscope operator. In the second case, reactive ductal cells were misinterpreted as atypical. There was only one diagnostic discrepancy in the 24 cases done prospectively with a concordance rate of 95.8%. This discrepant case was initially diagnosed as benign whereas the cell block showed metastatic renal cell carcinoma. Review of slides revealed misinterpretation of atypical malignant cells as being histiocytes.

Conclusions: Telecytopathology appears to be a reliable and effective method for rendering preliminary diagnosis and adequacy assessment during FNA procedures. It saves valuable time for the cytopathologists and makes real time consultation possible. Operator dependent mistakes can likely be overcome with further experience and training.

460 Paired Box Gene 8 (PAX8), HBME-1 and CK19 Expression in Preoperative Fine Needle Aspiration (FNA) of Papillary Thyroid Carcinoma: Diagnostic Utility

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Background: The diagnosis of papillary thyroid carcinoma (PTC) can be particularly challenging in FNA's with low cellularity. The use of immunohistochemical (IHC) stains, such as HBME1 and CK19, have been extensively studied to increase the diagnostic accuracy of PTC. PAX8, a member of the thyroid-specific transcription factors, has recently been investigated, mostly with molecular techniques, as a useful marker of thyroid epithelial neoplasms. Its utility by IHC in PTC has not yet been well studied. Our goal was to compare the IHC expression of PAX8 with HBME-1 and CK19 expression on cell block preparations from FNA samples with a diagnosis of PTC. To the best of

the authors knowledge this is the first study documenting the results of these markers as a panel, in thyroid FNA samples.
Design: 32 cases of PTC diagnosed on FNA were included. The FNA cell blocks were IHC stained for PAX8, HBME-1 and CK19. 10 cases of benign thyroid nodules (BTN) were included as a control group.
Results:

Table 1: Immunohistochemical staining results.

	PAX8	HBME-1	CK19
PTC	31/32 (96.8%)	25/32 (78.1%)	24/32 (75%)
BTN	10/10 (100%)	1/10 (10%)	1/10 (10%)

Table 2: Sensitivity and Specificity of IHC stains used.

	Sensitivity	Specificity
PAX8	0.97	0.10
HBME-1	0.78	0.90
CK19	0.75	0.90

Conclusions: PAX8 demonstrates positivity in a very high number of patients with PTC. However, its positivity in all cases of BTN limits its usage for detecting PTC in FNA samples. HBME-1 had the highest sensitivity and specificity for PTC in our study with CK19 demonstrating a high degree of both sensitivity and specificity for PTC. HBME-1, CK19 and PAX8 may all have a role, particularly as a panel, in detection and diagnosis of PTC. However, PAX8 demonstrates a very low specificity, which may limit its utility in detecting PTC.

461 Fine Needle Aspiration of Follicular Lymphoma: A Study of 109 Patients

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Background: The clinical value of fine needle aspiration (FNA) for the diagnosis of follicular lymphomas (FL) is often questioned since it is perceived that the FNA is usually followed by a confirmatory surgical biopsy. However, in our experience, therapeutic decisions for many patients with FL have been based solely on the FNA diagnosis.
Design: Our LIS was searched for the 19.5-year period ending July 8, 2009 and all cases in which FL was mentioned in the FNA or hematopathology (HP) reports were identified. All correlating FNA, HP and flow cytometry reports as well as all available clinical notes and selected microscopic slides were retrospectively reviewed.
Results: There were a total of 109 patients (111 cases) in this study, including 2 patients who each had 2 FNAs with 2 corresponding biopsies. Only 13 of our 109 patients (12%) had a concurrent HP biopsy at the time of the FNA. Of the 109 patients, 65 (60%) had an FNA diagnosis of FL that was not subject to HP confirmation and the FNA diagnosis was instrumental in determining the nature of further treatment. There were 27 FNA cases diagnosed as FL with HP follow-up and the FNA diagnosis was confirmed in 21 of 27 cases (78%). The 6 FNA discrepancies included: 1 case of Hashimoto's thyroiditis, 3 cases of diffuse large B-cell lymphoma (DLBCL), 1 case of B-cell lymphoma, not further classified and 1 case diagnosed as fibrous tissue with infiltrating lymphocytes. There were 19 cases of HP-proven follicular lymphoma that had not been diagnosed as FL by FNA. The FNA diagnoses for these 19 FL cases included: malignant lymphomas (ML) with various descriptors, that were incorrectly subclassified (9 patients, 47%), suspicious/highly suspicious for ML (4 patients, 21%), atypical lymphoid infiltrates consistent with ML (2 patients, 11%), lymphocyte population with abnormal flow cytometric findings (2 patients, 11%), rare atypical lymphoid cells (1 patient, 5%), and reactive lymph node (1 patient, 5%). The grade of FL as assessed on the FNA matched the HP grade in 12 cases of the 16 cases (75%) in which grade was mentioned in both the FNA and corresponding HP reports.
Conclusions: A diagnosis of FL was established by FNA in 92 of our 111 cases (83%) but the subtyping accuracy for those cases with HP follow-up was only 78% and grading accuracy was only 75%. Nonetheless, in 60% of our patients, treatment regimens appear to have been based only on the FNA diagnosis of FL. Given these results, the policy of basing treatment of patients with FL solely on the basis of an FNA diagnosis requires re-evaluation.

462 Utility of Repeat Thyroid FNA in the Management of Patients with Thyroid Nodules

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Background: In the new Bethesda System for reporting thyroid FNA, repeat FNA is recommended for an initial diagnosis of non-diagnostic or atypical cells/follicular lesion of undetermined significance. We therefore investigated the pattern of repeat thyroid FNA at our institution.
Design: We retrospectively reviewed all reports on thyroid FNAs and thyroidectomies submitted to the pathology department from 2006 to 2008 at our institution. All patients who had a repeated FNA of the same lesion and all who had both FNA and thyroidectomy in the same time period were identified.
Results: 2,717 FNA specimens were identified. 608 had thyroidectomies during the same time period. 291 patients underwent repeated FNA at least once for 301 thyroid lesions. Thirty had a second repeat and one had a third repeat. The main findings are listed below. (ND=non-diagnostic; Sub=suboptimal; B=benign; F=follicular lesion with or without cystic change that cannot be further categorized due to cellularity; MF=microfollicular; H=Hurthle cell; P=papillary carcinoma; Ind=indeterminate for malignancy that includes MF, H, or suggestive but not diagnostic for P; Sus=suspicious for malignancy; Pos=positive for malignancy).

Diagnostic category	Initial aspirates (%)*	Number (%) with at least one repeat	Diagnosis of first repeat (%)*	Diagnosis of second repeat (%)*	% of patients with surgery after initial diagnosis**
ND	316 (13)	132 (42)	53 (18)	4 (13)	9.2
Sub -B or F	495 (21)	81 (16)	61 (20)	12 (40)	13
Sub -MF+H+P	84 (3.5)	27 (32)	19 (6.3)	1 (3.3)	40
B	980 (41)	28 (2.9)	124 (41)	12 (40)	6.8
Atypical	89 (3.7)	21 (24)	11 (3.7)	1 (3.3)	49
Ind	216 (9.1)	11 (5.1)	20 (6.6)	0	73
Sus+Pos	205 (8.6)	1 (0.49)	13 (4.3)	0	69
Total	2385	301 (13)	301	30	23

*The difference in distribution among the initial, the first and the second repeat aspirates is statistically significant by chi-square with $P < 0.01$. **Surgery during the study period at our institution.

Conclusions: Patients with ND are most likely to undergo repeat aspirates followed by those with a Sub specimen but suggestive of a worrisome lesion, such as MF, H, or P, and then followed by those with an atypical diagnosis. Although 40% of repeat aspirates were benign, the repeat aspirates were less likely to be diagnostic of a lesions (whether MF, H, Sus or Pos) than the initial aspirates and at least as likely if not more likely to be ND or Sub. Patients with an initial atypical diagnosis are twice as likely to have surgery than to have a repeat (49% vs 24%).

463 Target UroVysion FISH for Urinary Specimens Using the Duet Imaging System

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Background: Cytology has a poor sensitivity for urothelial carcinoma, and molecular tests such as UroVysion FISH may aid in interpretation. Target (or Location Guided) FISH is an ideal tool for combining morphology (stained or immunolabeled cells) with FISH. Studies by Daniely et al, provide clinical evidence that the combined analysis of morphology and FISH has high sensitivity and negative predictive value for the detection of bladder cancer.
Design: The BioView Duet ™ imaging system was used to identify abnormal-appearing cells by cytology followed by UroVysion FISH. A preliminary study using Pap-stained slides followed by UroVysion FISH showed problems with cell recovery and stain interference with subsequent FISH. To reduce cell loss and stain interference, this study included 19 slides that were stained with May-Grünwald/Giemsa and not coverslipped. Target cells were identified by brightfield microscopy, followed by slide de-staining and UroVysion FISH, to detect amplifications of chromosomes 3, 7, and 17, and deletions of 9p21. Target cells were then re-located using Duet software and fluorescence microscopy, and probe signals were scored for cell recovery, target cell re-location accuracy, FISH signal strength, reproducibility, and correlation with cytology results.
Results: Brightfield, DAPI, and signal quality scored 2.95, 2.95, and 3.0 (possible 3.0), and reproducibility was 100%. Target cell re-location was highly accurate with target cells re-located to the microscope field center in all but one case, for which 86% of the target cells re-located to the field center. Average target cell recovery was 78%, with >85% recovery for slides showing no scrape marks by fluorescence microscopy. Other slides with visible scrape marks had a 63% cell recovery, with damage presumed to occur during coverslip removal after FISH hybridization. Correlation with urine cytology was 92.8%. One of three atypical cytology cases was FISH positive, which may provide support for clinical correlation and further patient follow-up.
Conclusions: The tools provided by the Duet system for Target FISH have potential for reflex testing, combined immunocytochemistry/FISH, and research applications. In our experience, setting up a limited fluorescence scan with targets selected interactively rather than by automated scan, appears to be the best approach for Target FISH.

464 Evaluation of a Gene Expression Microarray Assay To Determine Tissue of Origin in Body Fluid Specimens

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Background: Identification of the tissue of origin is a common challenge for cytology specimens since tumors with uncertain origin represent 5-10% of all new cancer cases. Cytologic body fluids are routinely obtained in the diagnostic workup of these cases. The Pathwork Tissue of Origin test was cleared by the FDA as an in vitro diagnostic device for frozen tissue, and a version of the test has been developed that works with formalin-fixed paraffin embedded (FFPE) tissue, but is not cleared by FDA. Here, we present initial results for the evaluation of this assay in body fluid specimens using both the thrombin and Cellient™ cell block methodologies.
Design: We retrieved 8 tumor-positive and 7 negative body FFPE fluid specimens processed with both thrombin and Cellient™(Hologic, Bedford, MA) cell block methods (30 total samples). Two negative body fluids and two non-cytology tumor samples were used as controls. RNA was extracted from the FFPE samples and assays were performed according to a standardized amplification protocol and hybridized to Pathchip™ microarrays (Pathwork Diagnostics, Redwood City, CA). A Tissue of Origin report was generated for each sample, and compared with that of the primary tumor site. In addition, results between the thrombin and Cellient cell block methods are compared.
Results: All 30 samples provided sufficient RNA of adequate quantity and quality for the Tissue of Origin assay. To test the FFPE Tissue of Origin assay in our laboratory, we analyzed RNA from 2 primary tumors (one frozen, one FFPE), which correlated with the gene expression pattern of the original primary tumor (kidney and lymphoma). Complete data on 2 samples (1 thrombin, 1 Cellient) negative for tumor showed good performance with the Pathchip array, passing all quality thresholds and producing reliable gene expression profiles consistent with inflammatory and mesothelial cells.
Conclusions: Our preliminary results show that it is possible to obtain gene expression profiles from FFPE body fluid cytology specimens using the FFPE version of the Pathwork Tissue of Origin test. The remainder of our samples will be analyzed to

determine the ability to detect tissue of origin and the results from the thrombin and Cellient methods will be compared. These preliminary results suggest that gene expression profiling could be a useful approach for body fluid cytology specimens.

465 Numerous, Fine, Clear, Cytoplasmic Vacuoles May Be a Helpful Cytologic Feature in the Diagnosis of Pancreatic Endocrine Neoplasm on Pancreatic Endoscopic Ultrasound-Guided Fine Needle Aspiration

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Background: Pancreatic Endocrine Neoplasm (PEN) and Solid Pseudopapillary Neoplasm (SPN) are difficult to differentiate on fine needle aspirates of the pancreas and therefore comprise a common differential diagnosis in fine needle aspiration (FNA) or endoscopic ultrasound guided fine needle aspiration (EUS-FNA). Both entities produce numerous single plasmacytoid cells on cytologic smears. We have previously reported that large clear cytoplasmic vacuoles (LCCVs) can serve as a clue to favor the diagnosis of SPN and also noted that fine, clear, cytoplasmic vacuoles (FCCVs) could be seen in PEN and SPN, but that these tended to be more numerous in PEN. The current study attempts to determine whether the presence of numerous FCCVs can discriminate between PEN and SPN.

Design: We retrieved all the cases for PEN (n=17), SPN (n=7), and metastatic renal cell carcinoma (n=2) from the teaching files of our institution (performed in the context of pancreatic EUS-FNA) and reviewed Wright-stained smears for the presence or absence of numerous FCCVs, defined as vacuoles that were 1) round cytoplasmic inclusions, 2) optically clear, 3) smaller in size than the nucleus of a red cell, 4) present in sufficient number such as to partially obscure the nucleus (usually greater than 10), and 5) present in a majority of cells in at least one high power field. FNA diagnosis was based on published cytologic criteria with immunohistochemical confirmation in all cases. Some, but not all, cases were confirmed on subsequent surgical excision.

Results: We found that numerous FCCVs, as defined above, were present in approximately half (8/17) of the PEN and none of the SPN (0/7), ($X2 = 4.941$; $p = 0.0262$). One caveat, besides the small number of SPN in this study is the possibility of metastatic renal cell carcinoma to the pancreas, which we found will also produce numerous FCCVs. Careful attention to other cytologic characteristics, particularly the lack of numerous single plasmacytoid cells, can help the cytologist avoid this pitfall. The possibility that the clear-cell variant of PEN, associated with Von Hippel-Lindau syndrome, may be overrepresented should also be considered.

Conclusions: These results are encouraging that the presence of strictly defined numerous FCCVs may be useful in discriminating PEN from SPN on Wright-stained smears on FNA and EUS-FNA.

466 The Utility of Napsin-A in the Identification of Primary and Metastatic Lung Adenocarcinoma among Cytologically So-Called "Poorly Differentiated Carcinomas"

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Background: Cytologic sampling of lesions plays a critical role in the diagnosing and staging of lung cancer, particularly in patients with advanced disease (stage III and IV). Currently, medical treatments for non-small cell lung carcinoma have advanced to the point at which further delineation of adenocarcinoma versus other carcinoma is necessary. Although lung adenocarcinoma can be differentiated and diagnosed in most of the cytological cases, a significant number of cases are still difficult to classify due to a variety of reasons. Napsin-A has recently come to attention as a potential marker of lung adenocarcinoma. Only a few studies have investigated the role of Napsin-A in daily cytology practice. In this study, we have investigated the utility of Naspsin-A, and compared its sensitivity with TTF-1, in the identification of lung adenocarcinoma from so-called "poorly differentiated carcinoma".

Design: A computer search of a large teaching hospital identified 41 cytology cases of "poorly differentiated carcinoma," each having adequate cell block material for immunohistochemical staining and surgical follow-up confirming poorly differentiated adenocarcinoma of the lung. Cases consisted of metastatic (n=24) as well as primary lung (n=17) lesions. Sites of metastasis included neighboring lymph nodes (n=15); pleural fluid (n=5); liver/other (n=4). Cases were subsequently stained for TTF-1 and Napsin-A. Staining characteristics were then correlated to determine the sensitivity of the immunostains for identification of lung adenocarcinoma.

Results: Concordant results were seen in 38/41 (93%) of cases. Among primary lung adenocarcinomas TTF-1 and Napsin-A were positive in 10/17 (59%) and 9/17 (53%) respectively, while 7/17 (41%) cases were negative for both markers. Within metastatic lung adenocarcinomas, TTF-1 and Napsin-A were positive in 17/24 (71%) and 15/24 (63%) respectively, with 6/24 (25%) cases negative for both markers. The overall sensitivity of TTF-1 was 66%, while Napsin-A was 59%.

Conclusions: TTF-1 remains a superior marker, as compared to Napsin-A, for the identification of primary and metastatic pulmonary adenocarcinomas, including those designated as poorly differentiated. However, as Napsin-A closely correlates with TTF-1 staining, it may be used as an accompanying marker in cytopathologic diagnosis, particularly in those difficult cases.

467 Cytology of EBUS-TBNA Versus Conventional TBNA in Diagnosing and Staging of Lung Cancers. A Retrospective Study with Histologic Correlation

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Background: Conventional endoscopic transbronchial needle aspiration (TBNA) is a common procedure to obtain samples for diagnosing and staging lung lesions. Recently, endobronchial ultrasound-guided transbronchial needle aspiration (EBUS-TBNA) has been developed, with increased use by clinicians. Clinical data suggests that EBUS-TBNA has higher sensitivity and specificity than blind conventional TBNA in staging lung cancers. The purpose of our study is to investigate cytological features and the cytological diagnostic accuracy of these procedures.

Design: A computer search of our hospital for transbronchial fine needle aspirations (FNA) revealed 188 EBUS-TBNA cases (308 lymph nodes; 47 lung lesions) and 74 TBNA cases (106 lymph nodes; 44 lung lesions) over14 month time period. Sixty-eight percent of the cases had corresponding surgical material to correlate.

Results: Patients' ages ranged from 17 to 89 years old. Male and Female ratio was 1:0.8.

Table 1. Summary of cytologic diagnoses in lymph nodes and lung lesions.

Diagnoses	**Lymph node FNA**		**Lung FNA**	
	TBNA	EBUS-TBNA	TBNA	EBUS-TBNA
Benign/Reactive	**40 (37.7%)**	**149 (48.4%)**	**8 (18.2%)**	**14 (29.8%)**
Granuloma	**16 (15.1%)**	**35 (11.4%)**	**3 (6.8%)**	**2 (4.3%)**
Malignancy	**18 (17.0%)**	**84 (27.3%)**	**26 (59.1%)**	**27 (57.4%)**
Lung Primary	**18**	**71**	**22**	**22**
AdenoCA	6	18	10	5
SCC	4	12	7	7
SCLC	8	14	2	5
PD CA	0	18	3	2
Carcinoid	0	1	0	2
Lymphoma	0	8	0	1
Metastases	**0**	**13**	**4**	**5**
Atypical cells	**0**	**2 (0.6%)**	**4 (9.1%)**	**3 (6.4%)**
Suspicious for CA	**2 (1.9%)**	**11 (3.6%)**	**2 (4.5%)**	**0**
Non-diagnostic	**30 (28.3%)**	**27 (8.7%)**	**1 (2.3%)**	**1 (2.1%)**
Total	**106 (100%)**	**308 (100%)**	**44 (100%)**	**47 (100%)**

SCC=squamous carcinoma; SCLC=small cell lung carcinoma; PD CA=poorly differentiated carcinoma; CA=carcinoma

Cytology-histology correlation revealed that both TBNA and EBUS-TBNA had similar diagnostic profiles for lung lesions. However, for evaluation of malignant lymph nodes, 4 out of 65 (6.2%) was missed by EBUS, and 9 out of 40 (22.5%) was missed by TBNA.

Conclusions: Our data showed that EBUS-TBNA had a higher accurate diagnostic rate than conventional TBNA for staging of lymph nodes. Our findings suggest that EBUS-FNA cytology is an optimal modality for diagnosing and staging in clinically suspected lung cancer patients.

468 Outcomes in High-Risk HPV-Positive and HPV18/18-Positive Women with ASC-US Differ Based on the Women's Age Group

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Background: By ASCCP guidelines, women with ASC-US on Papanicolaou tests (PT), undergo HPV DNA testing to determine high-risk HPV (hrHPV) infection. Colposcopy with biopsy is recommended for hrHPV+ patients. However, data on outcomes according to HPV genotyes and age are limited. The aim of this study was to determine the age-specific likelihood of CIN2/3 in follow-up biopsies of women with hrHPV+ ASC-US and further evaluate the use of HPV16/18 genotyping in ASC-US.

Design: We compiled cases of ASC-US with reflex HPV testing from 2003 to 2008. HPV infection was diagnosed by PCR using MY09/11primers and genotyping by RFLP. Follow-up biopsies up to 6 months from the index PT were included. The ASC-US diagnosis was made according to the 2001 Bethesda System. Women were divided into 2 groups by age, with 30 years as the cutoff.

Results: Of the 2910 women with hrHPV+ ASCUS, 1126 women aged 17-75 (mean 30+/-10) had follow-up histologic results (biopsy rate 38.7%). The biopsy rate was lower for women <30 than for those ≥30 (680/1852, 36.7% vs. 446/1058, 42.2%, p=.0039). Follow-up biopsies showed 371 CIN1 (33.0%), and 260 CIN2/3 (23.1%) results.

Table 1. Biopsy Follow-up on HPV16/18+ ASC-US Cases Stratified by Age

Age	**No. F/U Biopsies**	**No. CIN1 on F/U (%)**	**No. CIN2/3 on F/U (%)**
10-19	118	40 (33.9%)	43 (36.4%)
20-29	562	197 (35.1%)	138 (24.6%)
30-39	250	82 (32.8%)	51 (20.4%)
40-49	133	39 (29.3%)	20 (15.0%)
50-59	48	11 (22.9%)	6 (12.5%)
60-69	12	1 (8.3%)	2 (16.7%)
70-79	3	1 (33.3)	0 (0%)
Total	1126	371	260

While the rate of CIN1 diagnoses did not differ with age, the rate of CIN2/3 diagnoses was significantly lower in women ≥30 (26.6% vs. 17.7%, p=.0005). The rates of CIN2/3 in HPV16/18+ women <30 and ≥30 showed similar differences (133/415, 32.1% vs. 42/195, 21.5%, p=.0073).

Table 2. Follow-up of CIN 2/3 Detection Rate Among Younger and Older Women With HPV16/18+ and ASC-US

Age	**No. F/u**	**CIN 2/3 (%)**	***P* value**
<30	680	181 (26.6%)	0.0005
≥30	446	79 (17.7%)	0.0073
TOTAL	1126	260	

Conclusions: The rate of CIN2/3 diagnoses in hrHPV+ women of all ages with ASC-US was higher than reported by other similar studies (Armah et al. Arch Pathol Lab Med. 2009;133:1426–1430). Hr-HPV+ and HPV16/18+ women <30 with ASC-US had higher follow-up rates of CIN2/3. The role of HPV16/18 genotyping in women with ASC-US is uncertain and needs further studies.

469 Endobronchial Ultrasound-Guided Transbronchial Fine Needle Aspiration: A Sensitive and Specific Diagnostic Technique

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Background: Endobronchial ultrasound (EBUS) with transbronchial fine needle aspiration (TFNA) is being used increasingly for diagnosis of mediastinal/pulmonary lesions, and as a less invasive alternative to mediastinoscopy for sampling lymph nodes and staging patients with confirmed or suspected lung carcinoma. Relative to mediastinoscopy, the sample obtained with TFNA is small. The aim of the current study was to determine (1) the sensitivity and specificity of EBUS TFNA for neoplastic and non-neoplastic processes, and (2) the ability to provide specific diagnosis (e.g. small cell carcinoma (SCC), adenocarcinoma (ADCA), squamous cell carcinoma (SCCA), metastasis (METS) versus non-small cell carcinoma (NSCC)) to guide appropriate management.

Design: Computerized records and clinical follow-up of EBUS TFNAs spanning a nine-month period were reviewed. Cases were categorized as diagnostic (non-neoplastic and neoplastic) or non-diagnostic, and sensitivity and specificity were calculated. For the diagnostic neoplastic cases, availability of additional modalities (cell blocks and immunohistochemistry) contributing to specific diagnosis were assessed.

Results: A total of 49 EBUS procedures were performed, and follow-up data were available for 42 cases, which form the basis of the study. Of these 42 cases, 28 were diagnostic, including 2 non-neoplastic (granulomatous processes) and 26 neoplastic cases. There were 26 true positives (malignant), 4 false negatives, 12 true negatives, and 0 false positives. The sensitivity and specificity were 87% and 100%, respectively. Specific diagnoses of 1 SCC (4%), 12 ADCA (46%), 9 SCCA (35%), and 2 METS (8%), and non-specific diagnoses of 2 NSCC (8%) were rendered. Cell blocks and immunohistochemical studies were used for 23/26 and 16/26 neoplastic cases (respectively); cell blocks were also used for 1 of 2 non-neoplastic cases.

Conclusions: EBUS TFNA is a sensitive and highly specific procedure. Additionally, on-site assessment by the cytology staff is valuable for triaging specimens for cell blocks and additional subtyping of neoplasms. Overall, the results demonstrate EBUS TFNA is an effective and minimally invasive procedure with the ability to provide specific results with small samples.

470 Atypia of Undetermined Significance in Thyroid Fine-Needle Aspirations: Prognostic Value of Architectural and Cytologic Atypia

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Background: The atypia of undetermined significance (AUS) diagnostic category as defined in the 2007 National Cancer Institute (NCI) Thyroid Fine Needle Aspiration (FNA) State of the Science Conference is a heterogeneous category between benign and suspicious for malignancy. The clinical behavior of these lesions as a group is not well defined, nor is there significant data as to subgroup characteristics that may impart a higher risk of malignancy. Here, we report the prognostic significance of specific diagnostic features of AUS FNAs.

Design: All thyroid FNAs performed at Brigham and Women's Hospital from 1/05 to 4/09 with a diagnosis of AUS were analyzed retrospectively. As appropriate, our laboratory qualifies AUS as being due to cytologic or architectural atypia (or both). Follow-up data from repeat FNA or surgical resection were correlated with AUS qualifiers as well as any other diagnostic descriptors provided in the original report (atypia severity, specimen cellularity, number of atypical cells, presence of colloid, histiocytes, cyst lining cells, or Hurthle cell changes).

Results: A total of 306 thyroid AUS FNAs had conclusive follow-up data, stratified on the basis of atypia (see table). The risk of malignancy for architectural atypia alone (14%) was significantly lower than that observed for the other groups (Z=2.282, >95% CI): cytologic atypia (30%), both cytologic and architectural atypia (28%), or unspecified atypia (32%). No significant predictive value was noted for any of the other descriptive qualifiers, either with respect to AUS lesions overall or for any of the four atypia subcategories.

Outcome for thyroid AUS FNAs based on atypia qualifier

	Architectural	Cytologic	Both	Unspecified	Total
Benign	55 *(86%)*	38 *(70%)*	58 *(72%)*	73 *(68%)*	**224 (73%)**
Malignant	9 *(14%)*	16 *(30%)*	22 *(28%)*	35 *(32%)*	**82 (27%)**
Total	**64 (21%)**	**54 (18%)**	**80 (26%)**	**108 (35%)**	**306**

Conclusions: As a group, 27% of thyroid FNAs diagnosed as AUS with clinical follow-up are malignant. Our data indicate that the isolated presence of architectural atypia has approximately half the relative risk for malignancy compared to AUS with cytologic atypia, cytologic as well as architectural atypia, or with no further descriptor provided. Apart from these atypia qualifiers, no other diagnostic features were noted to carry significant prognostic weight in interpreting AUS FNAs, although case numbers were limited for many of these qualifiers. Further study is needed to establish whether distinct patterns of AUS warrant different clinical follow-up algorithms.

471 Atypia of Undermined Significance in Thyroid Fine-Needle Aspirations: Four Year Institutional Experience with Clinical Follow-Up of 306 Patients

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Background: The 2007 National Cancer Institute (NCI) Thyroid Fine Needle Aspiration (FNA) State of the Science Conference proposed a uniform classification scheme with six distinct diagnostic categories. We report our institution's experience with the atypia of undetermined significance (AUS) category for which limited follow-up data has been reported to date.

Design: A retrospective analysis of all thyroid FNAs at Brigham and Women's Hospital from 1/05 to 4/09 was performed. Cases were reported using a diagnostic system essentially identical to the 2007 NCI proposal. FNA diagnostic categories were: non-diagnostic, negative for malignancy, atypical cells of undetermined significance (equivalent to AUS), suspicious for a follicular/Hurthle cell neoplasm, suspicious for malignancy, or positive for malignancy. Follow-up results for AUS FNAs including repeat FNA and surgical resection are reported here.

Results: Of 4683 thyroid FNAs, 487 (10.4%) had a diagnosis of AUS. Conclusive outcome data was available for 306 distinct cases [74 had no follow-up, 48 had a repeat FNA with inconclusive findings (non-diagnostic, repeat AUS, or suspicious diagnosis) but no further follow-up, and 59 were repeat AUS following an initial AUS FNA]. With an initial AUS FNA, 73.2% were benign on follow-up while 26.8% were malignant (see table). A repeat FNA after an initial AUS resulted in a diagnosis of: benign (50%), AUS (29%), suspicious (16%), positive (2%), or non-diagnostic (3%). After two successive AUS FNAs, surgical outcome was benign in 55% of cases and malignant in 45% of cases.

Outcome for thyroid nodule FNA following an initial AUS diagnosis (n=306)

Benign	**224 (73.2%)**	**Malignant**	**82 (26.8%)**
Benign follicular cells FNA	119 (53.1%)	Papillary carcinoma	73 (89.0%)
Adenoma/nodular hyperplasia	85 (37.9%)	Follicular carcinoma	8 (9.8%)
Hashimoto thyroiditis	12 (5.4%)	Anaplastic carcinoma	1 (1.2%)
Papillary microcarcinoma	8 (3.6%)		

Conclusions: AUS is a useful category in thyroid FNAs for stratifying patient risk for malignancy and directing clinical management. Overall risk for malignancy with a single diagnosis of AUS is intermediate between that of a negative and suspicious FNA. Although the majority of nodules with an initial AUS interpretation are benign, 26.8% were found to be malignant. In most instances, repeat FNA following an initial AUS diagnosis yields more definitive diagnostic classification. The approach of a repeat FNA following an initial AUS diagnosis is warranted, as this algorithm spares many patients from unnecessary surgery.

472 Mucoepidermoid Carcinoma: A Cyto-Histologic Correlation with Special Emphasis on Diagnostic Cytologic Features

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Background: Mucoepidermoid carcinoma (MEC) is the most common malignant tumor of the salivary glands; the majority of MEC occur in the parotid resulting in accessibility to biopsy by fine needle aspiration (FNA). However, the histologic complexity and morphological variability of this tumor may lead to interpretative difficulties on cytologic examination. In this study we attempt to identify morphologic features that may be useful in the FNA diagnosis of MEC.

Design: The cohort included 22 cases of MEC with preoperative FNA. Cytology and histology slides and clinicopathologic features were reviewed; cases were assessed for the following: % cystic component, nuclear atypia, necrosis, extracellular mucin, mucus cells, intermediate cells, oncocytes, cells with foamy/clear cytoplasm, keratinized cells and lymphocytes.

Results: 21/22 (95%) of MECs arose in the parotid gland (average size 1.9 cm). On FNA 7/22 (32%) cases were diagnosed as consistent with and 5/22 (23%) as suggestive of MEC; 6/22 (27%) as salivary gland neoplasm and 4/22 (18%) no tumor seen. The histologic grade was low in 12/22 (55%), intermediate 9/22 (45%) and high in 1/22 (4%) cases; neural invasion in 4/22 (18%) and lymph node metastasis in 1/22 (4%) cases. The cystic component was ≥50% in 17/22 (77%) cases. The morphologic features prevalent in both histology and FNA specimens were: mucus cells (95%), cells with foamy/clear cytoplasm (73%), intermediate cells (86%), presence of extracellular mucin (91%) and lymphocytes (86%). Oncocytic cells were seen in 23% and keratinized cells in 14% cases. Interestingly, cases with oncocytic cells and lymphocytes were interpreted as favor Warthin's tumor on FNA.

Conclusions: Presence of mucus cells, histiocytic type cells with foamy/clear cytoplasm, intermediate cells and lymphocytes in a mucinous background are strong diagnostic indicators of MEC; the presence of oncocytic cells along with the above-mentioned features should not refrain from diagnosing MEC in FNA specimens.

473 The Diagnostic Value of New Preparatory Procedures for Bile Duct Smear Cytology

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Background: The usefulness of brush cytology, routinely performed during endoscopic retrograde cholangiopancreatography (ERCP) to assess biliary stricture, is limited by relatively low sensitivity (18-57%). We assessed two novel cell-yield procedures and evaluate their utility.

Design: Between October 2008 to August 2009, patients found to have a biliary stricture suspicious for neoplasia on ERCP have undergone brush sampling for cytology with a standard single use brush (Cytology brush, Microvasive, Boston Scientific). The cytology specimen is obtained as follows: 1) After brushing, the cytology specimen is immediately transferred to glass slides by smearing the cellular material from the brush directly on two slides.(conventional smear) 2) Following the smearing, the cytologist suspended the cut off head of the brush from a wire, and then directly centrifuged the brush in tissue-culture medium (1 min, 3000 rpm). After removing the brush, the medium was centrifuged again, and the cell pellet smeared onto 2 glass slides. (the brush washing specimen) 3) The remaining sheath and wire were also transferred to the cytological laboratory. The cytologist cut the sheath into every 5-inch segments, and put all of the pieces into the centrifuge tube, and then centrifuged (1 min, 3000 rpm). Thereafter, the contents in the sheath were collected, and were submitted for cytospin preparation. (the contents of the sheath tube) All samples were fixed in 95% ethanol, and stained with Papanicolaou method. Cytologic categories employed were: benign, malignant, suspected malignancy, and inadequate. To evaluate the cellularity of the samples, the number of epithelial cell clusters was evaluated on Papanicolaou stained slide using on image analyzing system (WinROOF, Mitani Co, Tokyo, Japan).

Results: Brushing cytology samples were obtained from 28 patients. Final diagnosis was established by surgery, biopsy, or follow up. The sensitivity, specificity, and accuracy were 60.9%, 62.5%, and 61.3% on conventional smear, 85%, 100%, and 89.3% using the brush washing specimen, and the contents of the sheath tube. The average number of epithelial cell clusters was 5980 on conventional smear, 9901 using the brush washing specimen, and 22592 using the contents of the sheath tube($p>0.01$).
Conclusions: Significantly higher cellular and diagnostic yields can be obtained using these novel methods.

474 A Modified NCI Classification Scheme for Diagnosis of Thyroid FNA Using Only Four Categories Improves Intra- and Interobserver Diagnostic Agreement

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Background: The diagnostic reproducibility of the 2008 NCI scheme for diagnosis of thyroid FNA has not been previously reported. Our recent study using the NCI scheme identified considerable overlap in malignancy risk estimates for the "follicular lesion of undetermined significance (FLUS)" and "follicular neoplasm (FN)" categories and the "suspicious for malignancy (Susp)" and "malignant" categories. A simplified 4 category scheme yielding non-overlapping malignancy risk estimates was proposed.
Design: Six cytopathologists participated in a blinded retrospective review of 60 thyroid FNAs (10 cases from each of the 6 NCI diagnostic categories). Each case had been diagnosed 12-18 months earlier by one of the participants using the NCI scheme. Slides were independently reviewed by the 6 cytopathologists and assigned an NCI category without restriction in the number of diagnoses per category. No clinical/imaging information was provided. Intraobserver agreement was evaluated by comparing the percent diagnostic agreement between initial and review diagnoses using the NCI and the simplified 4-category (unsatisfactory, benign, FLUS/FN, Susp/malignant) schemes. Fleiss' generalized kappa and interobserver agreement by category were estimated using the two schemes and online software (www.ccit.bcm.tmc.edu/jking/homepage).
Results: Percent intraobserver diagnostic agreement ranged from 33%-100% and 50%-100% using the NCI and the simplified schemes, respectively. The table compares interobserver agreement levels, by diagnosis, using the 2 schemes.

Comparison of Kappa Values for Thyroid FNA Diagnosis

FNA diagnosis	NCI scheme	Proposed simplified scheme
Unsatisfactory	0.8	0.8
Benign	0.6	0.6
FLUS	0.3	0.5
Follicular neoplasm	0.5	
Suspicious for malignancy	0.2	0.8
Malignant	0.6	
Fleiss' generalized kappa	0.6	0.8

0-0.4=poor agreement, 0.4-0.75=fair agreement, 0.75-1.0=strong agreement

Conclusions: The use of the NCI classification scheme yielded considerable intraobserver and interobserver diagnostic variability, with poor or fair agreement levels in the FLUS, Susp, benign and malignant categories. Use of the 4-category scheme improved intraobserver and interobserver agreement levels. As our previous study has shown that the 4-category scheme also provides non-overlapping malignancy risk estimates, modification of the 2008 NCI recommendations for diagnosis and reporting of thyroid FNA appears warranted.

475 Does High-Risk HPV Genotyping of Abnormal Anal Cytology Improve Detection of High-Grade Anal Intraepithelial Neoplasia (AIN)?

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Background: High risk (HR) HPV has been established as the major cause of AIN with HPV 16/18 estimated to cause 80% of anal carcinomas. The anal Pap smear is relatively sensitive to detect AIN but has low specificity for predicting and tends to underestimate the severity of AIN observed on biopsy. We sought to determine the prevalence of HR HPV genotypes in anal cytology samples and to assess HR HPV genotyping as an adjunct in diagnosis of individuals with abnormal anal cytology.
Design: 101 anal SurePath samples (28 negative, 25 ASC, 34 LSIL, 6 ASCH, 8 HSIL) from patients 21-74 yrs in age (mean 46.5 yrs; median 46 yrs) were studied. After a Pap stained slide had been prepared from each specimen, DNA was extracted from the residual sample and subjected to multiplex real time PCR utilizing the COMPLeTe Care HPV test (PRL, Overland Park KS) that detects HR HPV 16,18,31,33,35,39,45,51,52, 56,58,59,68,73, and 82. HR HPV genotypes detected in the samples were correlated with concurrent cytodiagnoses and with followup at 1-30 months (mean 3.8 mos; median 4 mos).
Results: HR HPV was detected in 82% of the 101 (50% negative, 84% ASC, and 100% LSIL and above) cases. Multiple (from 2-9) HR HPV genotypes were detected in 71% of the cases. The average number of HR genotypes detected per case increased with severity of cytodiagnosis. HPV 16, present in 44% of all cases and in 53% of the HR HPV+ cases, was the most frequent HR type detected in each diagnostic category. Detection of HPV 16 and 16/18 increased from 21% and 29% respectively in negative up to 75% in HSIL cases. The 66 cases with followup included 25 AIN 2/3 and 18 AIN 1. 20 of the 25 AIN 2/3 were preceded by HPV 16/18+ cytology. 46% of the HPV16/18+ LSIL cases and 22% of the HPV 16/18+ ASC cases showed AIN 2/3 at followup. No genotype predominated among the non16/18 HR HPV+ cytology samples that showed AIN 2/3 at followup.
Conclusions: -The overall high prevalence of HR HPV detected in 82% of anal cytology samples from persons at increased risk for AIN indicates that screening for HR HPV is not efficacious in diagnosis of AIN 2/3 in this population. -80% of the AIN 2/3 at followup were preceded by HPV 16/18+ anal cytology. 39% of HPV 16/18+ ASC/LSIL samples showed AIN 2/3 at followup compared to 18% of the non16/18 HR HPV+ and HR HPV- ASC/LSIL cases. Testing for HPV 16/18 may help select cases for aggressive management.

476 Evidence-Based Pathology: Low Cost-Effectiveness of CD3/CD20 Immunostains in Triage of Lymphoid-Rich Pleural Effusions

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Background: CD3/CD20 immunostains are often performed in the cytologic evaluation of lymphoid-rich pleural effusions (LR-PE). Most benign LR-PE are predominantly composed of T(CD3+) cells while most malignant LR-PE are of B(CD20+) cell lineage. As part of the effort to contain laboratory costs, there is increasing interest in applying principles of evidence-based pathology to the use of immunostains.
Design: 258 consecutive LR-PE in which CD3/CD20 immunostains had been performed on cell blocks were retrieved from our cytology files. LR-PE had been diagnosed as reactive lymphocytosis or lymphoma/leukemia (L/L) based on morphology and immunophenotype. The utility of CD3/CD20 in diagnosis of L/L was assessed and the percentage of cases in which L/L was initially diagnosed in the LR-PE was determined by correlating the cytological findings with clinical information and laboratory data including flow cytometry, peripheral blood counts, lymph node and/or bone marrow biopsy results that were available at sign-out.
Results: Of the 258 LR-PE (from patients 22-95 years of age), 196 (76%) were reactive lymphocytosis and 62 (24%) were L/L (37 B-cell, 10 CLL/SLL, 6 T-cell, 4 PEL, 4 multiple myeloma, 1 AML). There was a previous diagnosis of L/L, concurrent diagnostic tissue, clinical evidence of persistent, progressive, or recurrent disease, and/or marked peripheral lymphocytosis in 44 (71%) of the L/L cases. A first diagnosis of L/L was made in the remaining 18 (29%) cases, comprising 7% of the LR-PE and 0.3% of all pleural effusions evaluated in our cytology laboratory during the study period. In 16 of these cases (12 B-cell lymphoma, 3 PEL, 1 multiple myeloma), the neoplastic cells were large with high grade features that mandated full lymphoma workup. Marked peripheral lymphocytosis was indicative of L/L in 8 of the 10 CLL/SLL cases. In 2 cases, comprising 0.8% of LR-PE, concurrent CBC was not available and CD20 positivity led to further workup and diagnosis of CLL/SLL in the LR-PE.
Conclusions: Our findings suggest there are very few scenarios where CD3/CD20 immunostains are cost-effective in the selection of LR-PE that warrant workup for an initial diagnosis of L/L as the prevalence of CLL/SLL first diagnosed in LR-PE is <1% and large cell L/L can be suspected on morphology and/or clinical parameters. Atypical hematopoietic cells should be further evaluated irrespective of CD3/CD20 stains. The results underscore the feasibility of applying evidence-based principles toward cost-effective use of immunostains in cytology.

477 Insulin-Like Growth Factor II mRNA-Binding Protein 3 Is a Useful Marker To Distinguish Malignant Cells from Benign Mesothelial Cells in Serous Fluids

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Background: Insulin-like growth factor II mRNA-binding protein 3 (IMP3) is an oncofetal protein which plays important roles in varied critical biological processes, such as RNA trafficking and stabilization, cell growth, and cell migration. IMP3 is highly expressed in fetal tissue during embryogenesis but not in most adult tissues. Recent studies showed that IMP3 is over-expressed in a number of malignant tumors and that the expression of IMP3 is an independent prognostic factor for some malignancies. In addition, IMP3 can be used to distinguish benign from malignant lesions. Determining whether an effusion is malignant can be difficult in some cases. Therefore, we wanted to determine whether IMP3 might have diagnostic utility in the differentiation of benign mesothelial cells from malignant cells in serous fluids.
Design: Total 65 cases of pleural (30) and abdominal (35) effusion specimens with cell blocks were selected from the cytopathology files of the Department of Pathology at Creighton University Medical Center. The cytologic diagnoses of these cases included: adenocarcinoma (29), pseudomyxoma peritonei (PMP, 9), malignant mesothelioma (9), other malignancies (3), atypical cells (3), and negative for malignancy (12). Monoclonal anti-IMP3 antibody (Dako, clone 69.1, 1:200 dilution) was used to stain the tissue from the cell blocks and an isotype IgG2a was used as a negative control. The slides were reviewed by a senior pathology resident (J Wang) and a cytopathologist (C Deng), and IMP3 staining was graded as negative (no staining), weak (1+), moderate (2+), and strong (3+) positivity, based on the intensity of the cytoplasmic staining.
Results: Twenty-nine out of 29 (100%) adenocarcinomas were strongly positive for IMP3; 9 out of 9 (100%) malignant mesotheliomas were variably positive for IMP3 (1+ to 3+); 8 out of 9 (89%) PMP showed mild to moderate staining (1+ to 2+); 1 squamous cell carcinoma was negative; the other 2 malignant tumors were weakly and strongly positive respectively; 2 out of 3 cases with atypical cells were positive; only one of 12 (8%) benign fluids showed weak positivity for IMP3.
Conclusions: Our data indicates that IMP3 may be a useful marker to differentiate benign from malignant effusions. It may have particular diagnostic utility when atypical effusion cytology is encountered and the cells of interest do not possess clearly malignant features. Therefore, IMP3 should be included in an immunohistochemical staining panel to assist in distinguishing between benign and malignant effusions.

478 Over-Interpretation of Liquid-Based Pap Tests Due to Cervical Endometriosis: A Diagnostic Pitfall

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Background: Endometriosis of the cervix is a potential pitfall for the over-interpretation of cervico-vaginal Pap tests. This is further complicated by the variable morphologic features of cervical endometriosis, which are dependent on the patient hormonal status and menstrual cycle. The varied cytologic features overlap with both those of atypical

glandular cells and atypical squamous cells. As such, erroneous Pap test interpretations may range from atypical glandular cells (AGC) and atypical squamous cells, cannot exclude high-grade squamous intraepithelial lesion (ASC-H) to high grade squamous intraepithelial lesion (HSIL) and endocervical adenocarcinoma in-situ. The goal of this study was to examine the impact of biopsy-confirmed cervical endometriosis on the interpretation of the previous abnormal Pap test, in a large cytopathology laboratory.
Design: The Pathology computer files at our hospital were searched from 2004 to 2009 for patients with benign cervical tissue specimens containing endometriosis, which were preceded by abnormal Pap test results. HPV DNA test results, when performed, were recorded. All Pap tests were processed as ThinPrep, with HPV DNA testing performed by Qiagen HCII.
Results: Five patients were identified, ranging in age from 27 to 52 (mean 36). The prior Pap test diagnoses were: ASC-H (two cases), LSIL (two cases) and one case of AGC, favor neoplasia, which led to hysterectomy. Two patients underwent LEEP cone biopsy, one patient underwent cold-knife conization, and one patient had multiple cervical colposcopic biopsies. Histologically, four cases contained focal cervical endometriosis, with extensive endometriosis present in the remainder. No specimens contained evidence of either a squamous intraepithelial lesion or endocervical adenocarcinoma. HPV DNA testing was negative in four of the five patients.
Conclusions: Cervical endometriosis remains a cause of Pap test over-interpretation in a small but definite subset of patients. It is important to examine colposcopic/conization biopsies for this entity, particularly when dysplasia is not identified and HPV DNA testing is negative.

479 Atypical Ductal Epithelial Cells in Nipple Discharge: A Cyto-Histologic Correlation from a Series of 169 Cases

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Background: Nipple discharge is commonly associated with benign breast lesions; however, approximately 11% of cases are caused by malignancy. Cytological examination provides a quick and non-invasive method to evaluate patients with nipple discharge. The purpose of this study was to analyze our experience with atypical ductal epithelial cells (ADEC) in nipple discharge specimens in the diagnosis of benign and malignant breast lesions.
Design: Nipple discharge smears performed at our institution between 1990 and 2008 were retrieved from the archives of Cytopathology. Cytology findings were categorized as mildly ADEC, moderately ADEC, and severely ADEC suspicious for malignancy. The presence of blood was recorded on smears with mild or moderate ADEC. Follow-up in the form of surgical pathology was evaluated in all cases.
Results: 287 smears (16%) in a series of 1825 nipple discharge specimens contained ADEC over the last 18 years. Histological correlation was available in 169 cases (59%). The patients' ages ranged from 18 to 95 years old (mean age: 54) and 167 were females. Mildly ADEC were seen in 54 cases (32%), moderately ADEC in 99 cases (59%), and severely ADEC suspicious for malignancy in 16 cases (9%). Overall, of the total 169 nipple discharge specimens with ADEC, 121 cases (72%) had benign breast conditions (papilloma or fibrocystic change [FCC]) and 48 cases (28%) had carcinoma (in-situ or invasive). Of the 54 cases with mildly ADEC, 42 had benign lesions (78%; 29 papilloma, 13 FCC) and 12 had carcinoma (22%; 9 in-situ, 3 invasive). Of the 99 cases with moderately ADEC, 71 showed benign conditions (72%; 43 papilloma, 28 FCC) and 28 had carcinoma (28%; 19 in-situ, 9 invasive). Finally, of the 16 cases with severely ADEC suspicious for malignancy, 8 showed benign lesions (50%; 4 papilloma, 4 FCC) and 8 had carcinoma (50%; 4 in-situ, 4 invasive). The two male patients had papilloma and gynecomastia. A bloody background was noted in 2 smears with mildly ADEC (both had in-situ carcinoma) and 17 smears with moderately ADEC (10 had papilloma or FCC and 7 in-situ carcinoma).
Conclusions: Our experience revealed a papilloma or FCC rate of 72% and a carcinoma rate of 28% when ADEC are seen on nipple discharge smears. Our study shows that even when the atypia is considered mild, the rate of having a carcinoma is significant. Our study also shows that the presence of blood is associated with an increased rate of malignancy since 9 of 19 cases with mild/moderate ADEC and blood had a carcinoma (47%).

480 Prevalence of High-Risk Human Papillomavirus (HPV) in HIV-Positive Males Diagnosed with ASC-US in Anal Cytology Specimens

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Background: Human Immunodeficiency Virus (HIV)-positive men have an increased incidence of anal carcinoma and Human papillomavirus (HPV)-associated intraepithelial squamous anal lesions on anal cytology specimens. While HPV testing is extremely useful in triaging atypical squamous cells (ASC) of undetermined significance (ASC-US) in cervico-vaginal pap smears and reduces the number of unnecessary colposcopies, very little information exists regarding the prevalence of high-risk HPV (HR-HPV) in HIV-positive males with ASC-US on anal cytology. This study sought to determine if HPV screening is useful in triaging these patients by estimating the prevalence of HR-HPV in this cohort.
Design: We identified 43 HIV-positive males with an ASC-US result on anal cytology. Six patients were excluded because the HPV test results were equivocal or were not performed. A total of 37 specimens with corresponding HPV results were analyzed. All specimens were Thin Prep™-based and Papanicolaou-stained. HR-HPV testing was performed by hybrid capture assay (Digene®).
Results: Patients ranged in age from 25– 63 (mean=50 years). Overall prevalence of HR-HPV in the HIV-positive males was 62% (23/37). Sixteen of 23 patients in whom HR-HPV was detected had follow-up, either with cytology or biopsies. Eight of 16 patients with subsequent cytology specimens were diagnosed as follows; ASC-US (n=4), ASC-Cannot exclude a high-grade lesion (ASC-H) (n=1), low-grade squamous intraepithelial lesion (LSIL) (n=1) and high-grade squamous intraepithelial lesions (HSIL) (n=2). The remaining 8 patients had subsequent anal biopsies that were diagnosed as negative(n=4) and anal intraepithelial neoplasia (AIN) 1 (n=1), AIN 2 (n=2) and AIN 3 (n=1). Therefore, half of the patients with histological correlation showed the presence of AIN. Six of the 14 (43%) HPV-negative patients had follow-up, either with cytology or biopsies. Five of the 6 patients had subsequent cytology specimens that were diagnosed as follows; negative (n=4) and LSIL (n=1). The sixth patient had a subsequent anal biopsy showing AIN 1.
Conclusions: Anal cytology specimens containing HR-HPV are highly associated with AIN, 25% of which are high-grade on subsequent cytology/biopsy follow-up. HPV testing has a potential role in the triage or management of these patients and can reduce the number of unnecessary anoscopies performed.

481 The Distribution and Frequency of Abnormal Anal Pap Tests in HIV Patients

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Background: Anal Pap test has been increasingly used as a screening tool for HPV related lesions among HIV patiebts. It is reported using Bethesda terminology. However the diagnostic criteria and clinical utility in these samples are not well established. We have experienced a marked increase in anal Pap smear volume at our institute since the late 2007.
Design: A computer-based search was carried out to retrieve all anal Pap tests from May 2007 to August 2009. Patients' information, ThinPrep Pap test and HIV detection results were obtained from Copath system. Plasma HIV-1 RNA was determined by using HIV quantitative RT-PCR.
Results: 688 Anal Pap tests were performed during the study period. 51 (7.4%) were reported as unsatisfactory. 637 Anal tests were included in the study. 99% patients were male with more than 74% white people. The mean age was 45.7 yr (20-73). 90.7% were HIV-1 positive with 31% cases showing HIV genomic titer >50 copies/ml. Distribution of abnormal anal Pap was listed in the table 1.

General information and distribution of atypical anal Pap tests

	Negative	ASC-US	ASC-H	LSIL	ASC-H/LSIL	HSIL	Total
No. cases (%)	178 (27.9)	162 (25.4)	17 (2.7)	183 (28.7)	23 (3.6)	74 (11.6)	637
Mean age (range)	46 (20-73)	45 (20-68)	47.7 (37-57)	44.4 (20-70)	46.8 (35-62)	49 (22-69)	45.7 (20-73)
Sex. M/F	177/1	158/4	17/0	182/1	23/0	74/0	631/6
Race w/b/uk	139/32/7	119/28/15	17/0/0	119/38/26	20/3/0	58/11/5	472/112/53
HIV+ (%)	150 (84.3)	150 (92.6)	16 (94.1)	172 (94.0)	22 (95.7)	68 (91.9)	578 (90.7)
HIV title>50 (%)	33 (22.0)	50 (33.3)	3 (18.8)	64 (37.2)	9 (40.9)	21 (30.9)	180 (31.1)

Conclusions: Conclusion: 1. The rate of abnormal anal cytology in the high risk population is extremely high (72%) with LSIL and ASC-US cytology as the most common interpretation. 2. The distribution of abnormal anal Pap categories is different from abnormal cervical Pap categories. The ratio of ASC/SIL (0.70, 179/257) in anal Pap tests is much lower compared with cervical Pap tests. 3. HIV positive rate in patients with abnormal anal Pap tests is significant higher than that in patients with negative anal Pap tests (P<0.001). 4. The number of the cases with high viral loads in group with abnormal anal test is significant higher than that in normal anal Pap test group (P=0.005). HIV-1 positivity and viral loads have no difference among different abnormal Anal Pap groups. 5. The significance of these abnormal anal Pap tests in HIV patients will be demonstrated in a histological follow-up study.

482 EBUS-TBNA Cytology of PET Positive Verse PET Negative Lymph Nodes. A Retrospective Study with Histology Correlation

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Background: Positron emission tomography (PET) is a widely used procedure in the assessment of lymph node (LN) status in the staging of lung cancer patients. Clinically, a PET positive LN often requires histological confirmation of malignancy, since an increased FDG uptake may be related to non-neoplastic processes. Endobronchial ultrasound-guided transbronchial needle aspiration (EBUS-TBNA) is a recently developed technique for diagnosing and staging of the lung cancer. However, only a few studies have investigated the correlation between EBUS-TBNA cytology and PET scan findings. In this study, we have reviewed the EBUS-TBNA cytological features of PET positive LNs verse PET negative LNs.
Design: Cytological cases of EBUS-TBNA with PET scan were searched over a period of 12 months from a major medical center. A total of 105 cases were selected, including 129 PET positive and 48 PET negative LNs. Among 105 cases, 67 cases (63.8%) had corresponding surgical material which was also reviewed and correlated.
Results: The location of LNs were as follows: 3.1% of 2(L+R), 13.2% of 4L, 31.8% of 4R, 24.8% of 7, 27.1% of intrapulmonary. The size of PET positive LNs ranged from 0.5 to 3.9 cm with average of 1.2 cm, whereas the PET negative LNs ranged from 0.8 to 3.8 cm with average of 1.6 cm. The cytological diagnoses were summarized in Table 1.

Table1. Cytological diagnoses of lymph nodes.

Diagnoses	PET positive LNs	PET negative LNs
	Number (%)	Number (%)
Reactive	20(15.5)	19(39.6)
Granuloma	11(8.5)	7(14.6)
Malignant	75(58.1)	6(12.5)
Suspicious for ca	10(7.7)	1(2.1)
Non-diagnostic	13(10.1)	15(31.3)
Total	129(100%)	48(100%)

Conclusions: Approximately 60% of PET positive LNs were confirmed by EBUS as malignant, 24% of the PET positive LNs were reactive or granulomatous inflammation; the non-diagnostic rate of PET positive LNs was 10%, whereas approximately 10% of PET negative LNs were diagnosed as malignant by EBUS, approximately 55% of PET negative LNs were reactive or granuloma, and the non-diagnostic rate was 30%. Our data suggest that EBUS-TBNA is necessary to confirm the PET findings, and EBUS may pick up additional 10% of PET negative malignant LNs in the staging of lung cancers.