and II (TGFBR2) is clinically available for the diagnosis of connective tissue disorders. Marfan syndrome (MFS) is caused by mutations in FBN1 and TGFBR2, while Loeys-Dietz syndrome (LDS) is due to mutations in TGFBR1 or 2.
Design: A total of 223 ascending aortic resections from patients aged 60 or less were accessioned between April 2007 to Aug 2008 and their histopathology results were tabulated. Referral pattern to clinical geneticists and results of genetic testing were reviewed from the medical records in this cohort of patients.
Results: Histopathologic examination showed aortic dissection in 52 patients (23%), cystic medial degeneration (CMD) in 45 (20%), increase in mucopolysaccharide content of the media without elastic fragmentation in 85 (38%), normal aorta in 15 (7%) and atherosclerosis in only 26 (12%). Of these 223 patients, 24 (11%) patients with either aortic dissection or CMD were referred to clinical genetics for evaluation. Five patients met the clinical criteria for MFS and diagnosis confirmed with mutations in FBN1. Nineteen patients did not meet criteria for MFS or LDS and were tested for TGFBR1 and 2; in addition, 4 patients also had FBN1 testing. One patient each were found to have mutation in TGFBR1, TGFBR2 and FBN1. The common clinical findings in these 3 patients are dilation of the ascending aorta, high-arched palate and translucent velvety skin. The patient with TGFBR1 mutation (c.1136T>C) is a 40 year old female with aortic dilatation and CMD on histology. The second patient with TGFBR2 mutation (c.914T>A) had thoracoabdominal aortic dissection at age 52 and found to have an enlarged aortic root. There was moderate increase of mucopolysaccharide material in the aorta but not CMD. Likewise, the 43 year old female with FBN1 mutation (c.2714G>A) did not show CMD in the aortic specimen. Conversely, 16 patients with mild to severe CMD did not have mutations in TGFBR genes.
Conclusions: In this retrospective analysis of patients with ascending aortic aneurysm or dissection, mutations were found in 15% of patients evaluated by a clinical geneticist. Phenotypic expression overlaps in patients with FBN1, TGFBR1 and 2 mutations. CMD is a nonspecific finding in ascending aortic aneurysms and dissections and is not always present in those with inheritable connective tissue disorders.

349 Isolated Vasculitis of the Female Genital Tract

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Background: Vasculitis involving the female genital tract is rare. This can be an isolated manifestation or part of a systemic disease. Diagnosis requires clinicopathologic correlation and appropriate clinical testing to rule out systemic involvement.
Design: Surgical pathology files were reviewed from 1992 to 2007 to identify cases of vasculitis involving the female reproductive system. Electronic medical records were utilized to determine the presence or absence of systemic disease with a minimum follow-up period of 6 months.
Results: There were 14 cases identified from a total of 9,846 hysterectomies with or without salpingo-oophorectomies over a 16-year period for an incidence of 0.14%. Five patients were excluded because of a lack of adequate follow-up data. Nine patients were found with isolated vasculitis after a median follow-up of 48 monhts. These patients range in age from 46 to 76 years. Symptoms were localized and related to vaginal bleeding or pelvic mass. Four of these patients had concurrent malignancies, 3 with endometrial carcinoma and 1 with ovarian carcinoma. The most common associated benign lesion was leiomyoma. The cervix accounted for the most frequent site of involvement (78%), either alone in 5 patients or in combination with the uterine corpus in 2 patients. One patient had vasculitis in the mesovarium. The most common histologic pattern is that of a necrotizing vasculitis involving medium-sized muscular-type arteries demonstrated in 7 patients (78%). The lesions are typically segmental with fibrinoid necrosis. The predominant inflammatory infiltrates are lymphocytes that can be admixed with neutrophils and rarely eosinophils. In contrast to systemic polyarteritis nodosa, inflammatory lesions are generally found in the same stage. One patient had lymphocytic vasculitis without fibrinoid necrosis and another patient showed granulomatous vasculitis. None of these 9 patients received medical treatment after the diagnosis of vasculitis.
Conclusions: Vasculitis of the female genital tract is rare and most often an incidental finding that is limited to the cervix. In majority of cases, these are isolated vasculitis. Histologically, these cannot be distinguished from systemic vasculitides. There is absence of systemic symptoms at the time of diagnosis. Patients do not require systemic therapy.

350 Diagnosis and Typing of Cardiac Amyloidosis in Routine Clinical Specimens by Mass Spectrometry Based Proteomic Analysis

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Background: Cardiac amyloidosis is a frequent cause of restrictive cardiomyopathy and, if untreated, leads to cardiac failure and death. Treatment strategies target the underlying pathogenesis and often involve high risk approaches such as organ transplantation. Therefore accurate typing of amyloid is of great clinical significance. Unfortunately, immunohistochemistry (IHC), currently used for typing amyloidosis, is problematic due to non-specific staining in approximately half of the cases. Here, we describe a novel mass spectrometry based proteomic approach which can type amyloidosis with high sensitivity and specificity and overcome many of the problems associated with IHC.
Design: We studied 56 cases of paraffin embedded cardiac biopsies involved by amyloidosis and 4 cases of normal cardiac biopsies. Congo red positive amyloid plaques were laser microdissected, trypsin digested, and analyzed by nano-flow liquid chromatography electrospray tandem MS (LC-MS/MS). The resulting LC-MS/MS data was correlated to theoretical fragmentation patterns of tryptic peptide sequences from the Swissprot database using Scaffold. Peptide identifications were accepted if established at greater than 90.0% probability and protein identifications were accepted if established at greater than 90.0% probability and contained at least 2 identified spectra. The identified proteins were examined for the presence or absence of amyloid related peptides. IHC for immunoglobulin kappa (IGK) and lambda (IGL) light chains, transthyretin (TTR), serum amyloid A (SAA) was performed in 52 cases.
Results: In 53/56 cases studied, LC MS/MS identified the presence of a single amyloidogenic protein. 35 cases showed a peptide profile consistent with TTR, 15 cases with IGL, 2 cases IGK and 1 case with SAA. No amyloidogenic peptides were identified in normal cardiac stroma or muscle. Of the cases where IHC was performed, staining was considered to be diagnostic in 19 cases and inconclusive in 33 cases. In each case, the IHC confirmed LC MS/MS findings. In those cases where the IHC was non-contributory, additional clinical and pathological information supported the amyloid type assigned by mass spectrometry.
Conclusions: LC-MS/MS proteomic analysis provides a highly specific and sensitive method for diagnosis and classification of amyloidosis in cardiac biopsy specimens. The method is rapid and readily applicable in a clinical setting to paraffin embedded tissues and will greatly improve the diagnosis and clinical management of cardiac amyloidosis.

351 Cardiac Overexpression of CXCL10 Causes Spontaneous Leukocyte Infiltration but Not Cardiac Dysfunction

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Background: The essential role of chemokines in dilated cardiomyopathy (DCM) has been demonstrated by recent studies. We previously showed the upregulation of cystein-x-cystein (CXC) chemokine ligand 10 (CXCL10) in Coxsackievirus B3 (CVB3)-induced myocarditis, a major cause of DCM.
Design: To explore the contribution of CXCL10 to CVB3-induced myocarditis and associated DCM, we performed functional analyses using newly generated transgenic mice (Tg) that cardiac-specifically overexpress CXCL10.
Results: A transgenic mouse model with cardiac-specific overexpression of CXCL10 was generated. The cardiac specific upregulation of CXCL10 was confirmed by PCR, RT-PCR, *in situ* hybridization, and Western blot analyses. Cardiac-specific expression of CXCL10 resulted in spontaneous infiltration of $CD4^+$ T cell, $CD8^+$ T cell, and NK cell in perivascular and interstitial regions of the myocardium as compared to control wild type littermates by both real time qRT-PCR and immunohistochemical staining. The number of infiltrations was age-dependent, with the greatest number in older Tg mice, but barely any in four-week-old mice. Further, the expression levels of IFN-γ, and counterinflammatory IL-10 cytokine in Tg hearts were significantly elevated as compared to that in wild type mouse hearts, but the expression levels of the pro-inflammatory cytokines (TNF-α, IL-4, IL-5, IL-6, IL-12) were unchanged. Despite the presence of mononuclear cell infiltrations and limited mRNA upregulation of IFN-γ and IL-10 in the myocardium, there were no discernible pathological alterations in the hearts of Tg mice, as revealed by (i) cardiac troponin I levels, a serum marker of myocyte injury; (ii) echocardiography, a measure of heart ejection fraction; and (iii) heart mass/body weight.
Conclusions: These findings indicate that CXCL10 primarily directs T cells and NK cells to the myocardium, and is associated with minor defense immunity but is insufficient to cause cardiac dysfunction.

Cytopathology

352 Cytopathological Changes of Cervical Smears in Patients with Uterine Prolapse: A Major Pitfall for Sqaumous Intraepithelial Neoplasia (Cervical Dysplasia)

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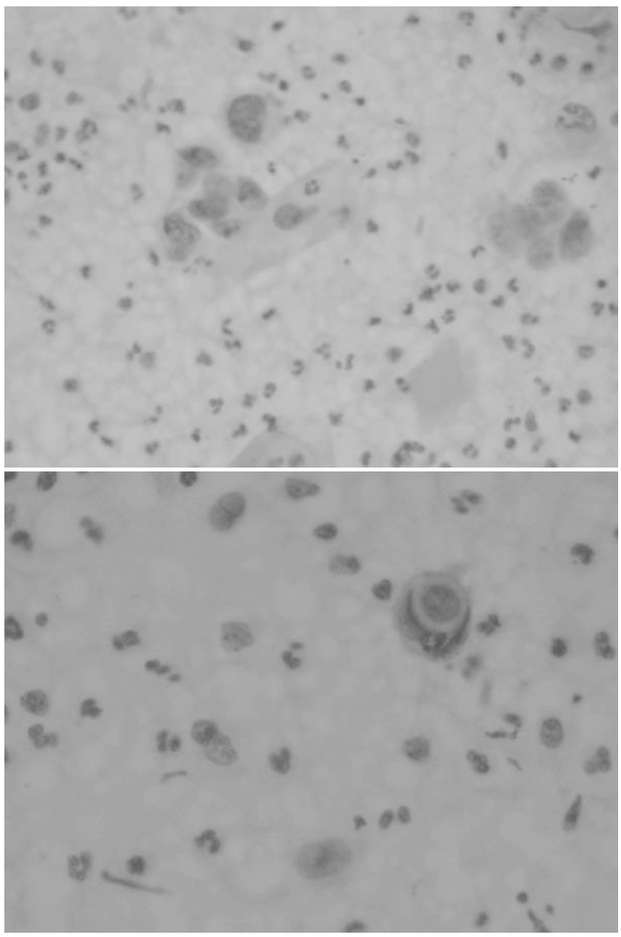
Background: Pitfall of cervical cytology is of utmost importance to be recognized due to its medicolegal consequences. Cytology of cervical smears from uterine prolapse patients can cause changes that may mimic dysplasia changes. Pathologists are likely to mistake these cases if encountered for the first time as dysplasia changes with Human papilloma virus (HPV)- like changes.
Design: Twenty cases of cervical swab from patients with uterine prolapse were studied. All patients had consequent simple hysterectomy due to prolapse symptoms. The patients' age ranged from 35-72 years of age with parity ranged from 4-14 kids.
Results: Cervicovaginal smears showed changes that are seen in cervical dysplasia: increased nuclear cytoplasmic ratio, nuclear membrane irregularities and perinuclear halos in 20/20 cases; discohesiveness of cells in 15/20 cases, cell hugging in 14/20 cases; nuclear hyperchromasia in 11/20 cases.



The followings changes are not seen in cervical dysplasia (but are seen in this entity): frayed cytoplasmic borders, neutrophilic debri in the background and the presence of vesicular nuclei (at least focally) in 20/20, and prominent nucleoli in 4/20 cases. Screening by PCR for Human papilloma virus (HPV) from cervical swab was performed on all these cases and proved to be negative.

Conclusions: Cytopathological changes of cervical smears in patients with uterine prolapse can be abnormal and may mimic cervical dysplasia. The cytological criteria that help in differentiating this diagnosis from dysplasia are: frayed cytoplasmic borders, neutrophilic debri in the background, vesicular nuclei in many of the atypical cells and occasionally prominent nucleoli. Awareness of these changes by the pathologists is very important to avoid falling into a mistake with its consequent medicolegal litigations.

353 Tissue Processor Protocol for Cytologic Preparations Improves Immunohistochemistry Results

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Background: Immunohistochemistry (IH) on touch and cytospin preparations is not commonly utilized because the procedure is usually not standardized for them and the results often do not correspond to those expected or to those observed in formalin-fixed paraffin-embedded tissue sections. We sought to determine whether the sensitivity and reliability of IH on cytologic preparations might be improved if they are subjected to pre-staining conditions similar to those used for formalin-fixed paraffin-embedded tissue.

Design: We obtained 2 sets of 45 air-dried touch or cytospin preparations from various tumors (n=12), body fluids (n=3) or normal tissue (n=3). One member of a pair was placed in a metal slide holder accompanying formalin-fixed tissue cassettes for routine overnight processing while the other was kept unfixed at room temperature. The following morning, IH was performed on the slide pair using an automatic immunostainer and stained with 1 of 26 antibodies commonly used to detect intermediate filaments and hematologic, neural, oncologic and other antigens. The antibody selected for each slide pair was one in which the corresponding antigen was anticipated to be present in that particular tumor type, body fluid or normal tissue. The slides were examined microscopically in a double blind fashion and semiquantitatively assigned to a quartile depending on the percentage of immunopositive cells. The staining intensity was graded as 0-3.

Results: Within the slide pairs, 58% of the tissue processor slides had a greater percentage of immunoreactive cells, 35% an equal percentage, and 7% a lesser percentage. Also, the staining intensity was greater in 56% of the tissue processor slides, identical in 40% and less in 4%. In both tumoral, body fluid and normal tissue specimens, the results in the tissue processor cytologic preparations were judged to be similar to those anticipated or observed (when available) in formalin-fixed paraffin-embedded tissue sections.

Conclusions: Subjecting cytologic preparations to tissue processor conditions helps equalize the pre-staining influences so that the IH protocol used for formalin-fixed paraffin-embedded tissue can be reliably applied to cytologic preparations. The cytologic IH results are similar to those observed in formalin-fixed paraffin-embedded tissue.

354 ASC-H: HPV Status and Histologic Correlation Based on Age Distributions

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Background: ALTS study has shown that age distribution is an important factor in disease outcome and application of reflex HPV DNA testing results for ASCUS cytology. However, assessment of the impact of age distribution on disease outcome and on HPV testing on atypical squamous cells, cannot exclude HSIL (ASC-H) is limited in the literature. We reviewed our experience over the last 5 years on ASC-H cytology and correlated age distribution with HPV status and histopathologic followup in a large cohort study.

Design: All cases with cytologic diagnosis of ASC-H between February 2003 and December 2007 were retrieved from our pathology database. Results of reflex HPV tests using the Hybrid Capture 2 (HC-II) method were tabulated. Histopathologic diagnosis from either biopsy or LEEP specimen was reviewed, when available. The most severe histopathologic diagnosis was recorded and HPV test results were correlated with both cytologic and histopathologic diagnoses based on age distribution.

Results: Our patients with ASC-H had ages ranging from 16 to 88, with 42% women younger than 30 years and 58% older than 30 years. Among 648 cases diagnosed as ASC-H, 95.5% (619) had HR-HPV test results, including 36.5% HPV negative and 63.5% HPV positive. HPV positivity of ASC-H gradually decreased from 95% in women <20 to 36% in women >51 years. About 77% (497) of cases had histologic follow up. Correlation with histology revealed that CIN2+ lesions were found in 41% of all ASC-H cases. Approximately 92% CIN2+ cases were HPV positive and 8% were HPV negative. Among HPV negative CIN2+ cases, 25% were seen in women >51 and 62.5% seen in women >31 years. Accuracy in detection of CIN2+ by ASC-H gradually decreased from 58% in women <20 to 18% in women >51 years. The majority of postmenopausal women with ASC-H were HPV-negative with atrophic change.

Conclusions: The specificity of cytologic diagnosis of ASC-H for detection of CIN2+ lesions is good in young women, but significantly decreased in postmenopausal women due to atrophy. Reflex HPV testing on ASC-H cases has a sensitivity, specificity, and negative predictive value of 94%, 30% and 89% respectively in women <30 years, compared to 71%, 64% and 93% respectively in women >51 years. The majority of HPV negative CIN2+ lesions was seen in women older than 30 years.

355 HPV Status and Histologic Follow up on Women with Cytologic Abnormality of ASC-H

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Background: Reflex HR-HPV testing for ASCUS has improved the specificity for detecting CIN lesions. However, studies examining the significance of reflex HPV testing on atypical squamous cells, cannot exclude HSIL (ASC-H) are limited. We reviewed our ASC-H cytology cases for the past 5 years to correlate ASC-H with HPV status and histopathologic followup in a large cohort study.

Design: All cases with cytologic diagnosis of ASC-H between February 2003 and December 2007 were retrieved from our database. Results of reflex HPV tests using the Hybrid Capture 2 (HC-II) method were tabulated. Histopathologic diagnosis from either biopsy or LEEP specimen was reviewed when available. The most severe histopathologic diagnosis was recorded and HPV test results were correlated with both cytologic and histopathologic diagnoses.

Results: Of a total 302,363 PAP tests performed, 648 cases (0.2%) were diagnosed as ASC-H cytologically. Of those, 619 cases (95.5%) had undergone reflex HPV testing and 497 (77%) had histopathologic diagnosis either by biopsy or by LEEP procedures. There were approximately 64% HPV positive and 36% HPV negative cases. Histopathologic examination revealed 42% benign cervix, 17% CIN1 and 41% CIN2+ lesions including 5 cases of AIS and 5 cases of invasive SCC. Of HPV positive cases, CIN2+ lesions were found in about 60% of cases, CIN1 in 19% and benign cervix in 21%. In contrast, among HPV negative cases, CIN2+ lesions were seen in about 8% of ASC-H cases, CIN1 in 12% and benign cervix in 80%. There is a statistically significant difference between the HPV positive and HPV negative groups both in detection of CIN 1-3 ($P<0.0001$) and CIN2+ lesions ($p<0.0001$). A total of 54 cases were diagnosed as ASC-H plus atypical glandular cells (44 AEC, 6 AGC and 4 AEM), which consists of about 68% HPV positive and 32% HPV negative. Followup study revealed 24 cases of CIN2+ including 2 AIS, 5 CIN1 and 24 benign cervix. CIN2+ lesions were found in 70% of HPV positive compared with 13% of HPV negative cases.

Conclusions: Reflex HPV testing has a high sensitivity (93%) in detection of high-grade cervical dysplasia among ASC-H patients. The odds ratio in detection of CIN2+ lesions is 8 times higher in HPV positive ASC-H than HPV negative ASC-H. Our study indicates that reflex HPV test significantly increases the specificity of high grade cervical dysplasia with cytologic diagnosis of ASC-H.

356 The Contribution of Fluorescence In-Situ Hybridization (FISH) Studies to the Fine-Needle Aspiration Biopsy (FNAB) of Soft Tissue and Bone Neoplasms

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Background: The diagnosis of soft tissue and bone neoplasms by fine-needle aspiration biopsy (FNAB) can be challenging due to overlapping cytomorphologic features. Molecular studies can be particularly helpful to render a specific diagnosis by detecting characteristic chromosomal translocations, or to provide prognostic or therapy-related information to the clinician. The goal of this study is to determine if fluorescence in-

situ hybridization (FISH) studies performed on cytologic material can be useful in the diagnosis and management of soft tissue and bone lesions.
Design: Between January 2004 and December 2007, 862 cases of soft tissue and bone were diagnosed by FNAB at our institution. FISH studies were performed in 85 cases (10%). We retrospectively reviewed these cases to see if the outcome of the FISH studies had additional value to the cytomorphologic analysis and other ancillary studies. The FISH studies were primarily performed on unstained direct aspirate smears, or occasionally on cell block sections and the results were classified as positive, negative, or inadequate for diagnosis.
Results: The 85 cases with FISH studies included 42 cases of hematopoietic tumors, 25 cases of mesenchymal tumors, and 18 cases of metastatic breast carcinoma. The indications for performing FISH studies were for tumor subclassification (67 cases, 79%) and detection of HER2/neu gene amplification (18 cases, 21%). The most common requested FISH studies were the IgH gene rearrangement for hematopoietic malignancies and t(12;16), t(12,22)/CHOP-TLC and t(11;22)/EWSR1 for mesenchymal malignancies. Of the 85 cases, FISH was positive in 37 (44%) cases, negative in 45 (53%) cases and inadequate in 3 (3%) cases. Of the 67 cases submitted for further tumor classification, 32 cases (48%) were successful in subclassifying the tumors. Of the 18 cases of metastatic breast carcinoma submitted for HER2/neu FISH, 16 cases (19%) were successful in determining the HER2/neu status.
Conclusions: This study illustrates that FISH studies performed on cytologic material can be advantageous as an ancillary technique in the diagnosis and management of soft tissue and bone lesions. Therefore, the preparation of adequate unstained smears or other material for the studies can be important when the FNABs are performed.

357 Evaluation of Diagnostic Efficacy of Image Guided Fine Needle Aspiration of Pancreatic Lesions. A Retrospective Study of 296 Patients

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Background: Cytology is an important diagnostic tool, used routinely for the diagnosis of pancreatic lesions. In this study we reviewed the fine needle aspiration biopsies (FNAB) of the pancreas and determined it's diagnostic accuracy.
Design: From our institutional data base, 296 pancreatic FNABs from Jan 2006 to Jan 2008 were retrospectively reviewed. The cases were analyzed to determine the nature of the lesion (solid/cystic), the cytological diagnosis (Dx), molecular analysis, and the histological follow-up (F/U). Sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) for the cytology results were calculated using the histological Dx as the gold standard.
Results: The cytological Dx of the 296 pancreatic FNABs were: 126 malignant, 8 suspicious, 60 atypical, 74 negative, and 28 unsatisfactory. There were 78 FNABs (26%) s with histological F/U and the cytological Dx for these cases were: 50 malignant, 6 suspicious, 9 atypical, 8 negative, and 5 unsatisfactory. Histological Dx was malignant in 48 of 50 cases (96%) diagnosed by FNAB as malignant, 5 of 6 cases (83.3%) diagnosed as suspicious, 7 of 9 cases (77.8%) diagnosed as atypical, and in 12 of 13 cases (92.3%) diagnosed as negative or unsatisfactory. Of the 5 false positive cases (FNAB of at least atypical), 2 had no representative material on surgical biopsies, one was a small lesion (7mm), and one had inadequate material on FNAB. Ten of 12(83.3%) false negative cases were cystic lesions. Correctly diagnosed lesions by FNAB include: 100% of adenocarcinomas, 93% of neuroendocrine neoplasms, 50% of mucinous neoplasms (including intraductal papillary mucinous neoplasms), in addition to schwannoma, solid pseudopapillary tumor, and metastatic renal cell carcinoma. A total of 41 cases (14%) had molecular analysis. Of the these, 33 (81%) were cystic and 16 (48%) of these were positive for a KRAS mutation and 8 (24%) had loss of heterozygosity. Overall the pancreatic FNABs showed sensitivity of 86% and specificity of 55%. The PPV and NPV were 93.5% and 33.3%, respectively.
Conclusions: In our experience, FNAB of pancreatic lesions is a sensitive diagnostic tool with a high PPV, and is particularly helpful for solid lesions. The majority of false negative lesions were cystic mucinous neoplasms, which are extremely difficult due to paucicellular specimens and gastrointestinal contamination, and therefore, other ancillary diagnostic tools (molecular analysis) should be considered if a cystic lesion is suspected radiographically.

358 Co-Analysis of HPV DNA, mRNA E6/E7 HR-HPV Expression and mcm2 and TOPIIa (ProEx C) in Cervical Pre-Cancer

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Background: The Pap smear introduced for cervical cancer screening has significant limitations in relation to sensitivity and reproducibility, despite recent advances in liquid based technology.
Design: In this study we examine the utility of using combined HPV DNA, mRNA and ProEx C analysis in cervical screening. 93 individual cases were examined using ThinPrep (LBC) preparations. These included: 26 negative smears, 39 ASCUS/LSIL smears, 22 HSIL smears and 6 glandular abnormalities. Aberrant S-phase induction was detected using ProEx C immunocytochemical test, which detects mcm2 and Topoisomerase II alpha. Intensity of staining was scored on a 0-3+ scale. HPV DNA status was established using Digene HC-2 HR HPV analysis. The expression of HR-HPV E6/E7 mRNA transcripts was examined using Norchip PreTect HPV Proofer assay, which detects E6/E7 transcripts to HR- HPV types 16, 18, 31, 33 and 45. Sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) were calculated using the Pap test as gold standard. Spearman correlation co-efficient was calculated to demonstrate correlation of each test; ProEx C, HC-2, PreTect HPV-Proofer to the Pap test.
Results: Mcm2 and Topo IIa staining positivity and intensity increased with grade of CIN. HPV DNA positivity was significantly more positive in benign and lower grade abnormalities than the HPV mRNA, which is in keeping with previous published studies. HPV mRNA increased with grade of CIN. The sensitivity of ProEx C was 52%, HC-2 was 89% and PreTect HPV-Proofer was 40%. The specificity for ProEx C was 85%, HC-2 was 80% and Pretect HPV-Proofer was 100%. The PPV for ProEx C was 90%, HC-2 91% and PreTect HPV-Proofer 100%. The NPV for ProEx C was 40%, HC-2 was 76% and Pretect HPV-proofer 42%. When ProEx C was negative, it correlated to 90% of Pap results. When ProEx C was positive it correlated to 91% of Pap results. When HC-2 was negative correlation to Pap was 93%; when HC-2 was positive, correlation to Pap was 88%. When PreTect HPV-Proofer was negative it had an 89% correlation to Pap, when positive it had a 93% correlation to Pap.
Conclusions: The findings suggest that combinational analysis using HPV mRNA and ProEx C will add significantly to prognostic stratification of women with cervical pre-cancer disease.

359 Do Classic Preparations of Gastrointestinal Cytology Perform Differently Than ThinPrep® Cases? Observations from the College of American Pathologists Interlaboratory Comparison Program in Nongynecologic Cytology (CAP NGC)

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Background: Liquid-based preparations (LBP) differ morphologically from traditional slide preparations and their use is becoming more common in examination of gastrointestinal (GI) cytology specimens. Discordant participant responses from challenges distributed in the College of American Pathologist's Interlaboratory Comparison Program in Nongynecologic Cytology (CAP NGC) were analyzed.
Design: Malignant ThinPrep® challenges distributed between 2000-07 were compared to classical preparations (smears, cytospins) for discordant responses (negative or unsatisfactory). Differences between preparation type, participant type, and diagnosis were analyzed.
Results: Classic preparations comprised 93% (n=11588) of the GI challenges while 7% (n=912) were ThinPrep® preparations. An exact match to the reference diagnosis of positive-malignant was seen in 88.5% of conventional preparations and 95.9% of ThinPrep (P<.001) challenges. These results were statistically significant when the specific reference diagnosis was adenocarcinoma (P<.001) but no difference for squamous cell carcinoma (P>.99). Cytotechnologist overall performance was not different compared to pathologists (89.2% v 89.0%; P=.75). For specific reference diagnosis, there was statistically better performance by cytotechnologists for cases of squamous cell carcinoma (96.3% vs. 92.6%; P<.001) and better performance by pathologists for cases of spindle cell neoplasm (79.7% vs. 42.9%; P<.001). No performance difference between participant types existed for adenocarcinoma (88.4% vs. 88.0%; P=0.637), carcinoid (83.7% vs. 67.3%; P=.06) or carcinoma NOS (52.9% vs. 66.7%; P=.19).
Conclusions: ThinPrep® performed significantly better than classic preparations of GI cytology specimens. Performance varied by reference interpretation, with adenocarcinoma performing best. Cytotechnologists and pathologists perform the same overall.

360 Interinstitutional Consultation in Fine Needle Aspiration Cytopathology

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Background: The importance of interinstitutional consultation has been documented across a variety of surgical pathology organ systems. Few studies exist regarding this practice within cytopathology, specifically, fine needle aspiration cytology (FNAC).
Design: All FNAC cases between 9/02 and 1/07 were reviewed. Original and 2nd opinion diagnoses were categorized as: no diagnostic disagreement, minor diagnostic disagreement, or major diagnostic disagreement, with the latter defined as either a 2-step deviation on a scale of "unsatisfactory, benign, atypical, suspicious, and malignant" or a change in treatment and/or prognosis. Outcome was determined by review of the electronic medical record.
Results: 742 outside FNAC cases showed minor disagreements in 132 cases (17.8%), and major disagreement in 69 cases (9.3%) with follow-up available for 60/69 major discrepancies. The 2nd opinion diagnosis was better supported upon follow-up in 65% and the initial diagnosis better supported in 33% of major discrepancies. However, 55% of cases where the original institution diagnosis was better supported were ones where either the entire case slides were not received, or slides were deemed extremely hypocellular and non-diagnostic on 2nd opinion. A 2nd opinion diagnosis prompted change in clinical management in 32/742 (4.3%) of cases. Aspirates most prone to change in management or therapy were from: thyroid (12), lymph node (9), salivary gland (4), and liver (2). Of 60 major diagnostic disagreements, board certified cytopathologists rendered 2nd diagnoses in 44 cases, with 75% better supported by follow-up while pathologists not board-certified in cytopathology had only 38% of 2nd opinion diagnoses supported.
Conclusions: Interinstitutional review of FNAC cases resulted in major discrepancies in 9.3% and a change in clinical management of 4.3% of cases. Fine needle aspiration presents unique diagnostic challenges, and review by a board-certified cytopathologist may lead to further error reduction.

361 On-Site Adequacy Assessment and Preliminary Diagnosis of Fine-Needle Aspiration Specimen by Telecytopathology

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Background: On-site evaluation of fine-needle aspiration (FNA) specimens by a pathologist is essential to obtain adequate samples and provide a preliminary cytologic diagnosis. However, distance from the pathology laboratory and multiple locations for FNAs makes pathologist on-site evaluation difficult. This study will summarize our experience of on-site evaluation through telecytopathology.

Design: Cytology smears were prepared by cytotechnologists and cytopathology fellows on-site. Dynamic images were captured and processed with Nikon Telepathology L2 System and transmitted via Ethernet, which was accessible from any computer with internet access. A pathologist interpreted the cytology images on a computer screen, communicated with on-site operators over telephone, and provided adequacy assessment and preliminary diagnosis. Rate of sample adequacy and accuracy of preliminary diagnosis through telecytopathology were compared with those obtained by conventional on-site method prior to the use of telecytopathology.

Results: Evaluation of 119 cases via telecytopathology and 144 consecutive cases with conventional pathologist on-site evaluation were compared. The specimen sites were similar in these two groups, included assessment of pancreas, lymph node, liver, stomach, duodenum, bile duct, esophagus, adrenal gland, and rectal sites. Rate of sample adequacy in the telecytopathology group and conventional group was 91.4% and 94.5%, respectively. The preliminary diagnoses of unsatisfactory, adequate (without specific diagnosis), negative/benign, atypical, spindle cell or neuroendocrine neoplasm, suspicious, and positive for malignancy were 8.6%, 21.0%, 17.6%, 12.6%, 5.9%, 5.0% , 29.4% in telecytopathology group and 5.6%, 32.6%, 22.2%, 9.7%, 2.1%, 6.3%, 22.2% in conventional group. The discrepancy between the preliminary and final diagnosis was 6.7% and 4.9%, respectively for telecytopathology and conventional groups. The major difficulty in telecytopathology was to distinguish reactive lymphocytes from neuroendocrine neoplasm of pancreas.

Conclusions: The study demonstrated that on-site evaluation of FNA specimens via telecytopathology provided similar results in assuring sample adequacy and preliminary diagnosis accuracy when compared with the conventional method. Telecytopathology allows pathologists use their time more efficiently and makes on-site evaluations at multiple and/or remote locations possible.

362 HPV Profile of Women in Belize City, Belize: Correlation with Cervical Cytopathologic Findings

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Background: Cervical carcinoma is the most common cancer among Belizean women; however, data regarding the frequency of human papillomavirus (HPV) genotypes and their association with cervical cancer and intraepithelial neoplasia are non-existent. We therefore included HPV genotyping as part of a cervical cancer screening campaign conducted in Belize City in 2007.

Design: An educational program was followed by routine gynecologic examination with colposcopy and further treatment when necessary. Conventional Pap smears with Hybrid Capture (HC) 2 HPV testing were performed on 463 women. All HC2-positive samples were genotyped using a developmental GP5+/GP6+ PCR-coupled Luminex assay for 2 low-risk and 18 high-risk HPV types.

Results: 8% of Pap smears were abnormal. The prevalence of high-risk HPV was 15.6% in the total population, 10.1% in those with normal cytology (92% of the total group), and 93.3% in women with a high-grade squamous intraepithelial lesion (HSIL), (3.2% of the total group). 31.9% of patients with HPV infections had multiple types (5.0% of the total group). 5.0% of all women and 2.6% of women with normal cytology had HPV16 or 18. For all women, HPV16, 18, 56 and 52 were present in decreasing order of frequency. HPV11 was present in only one patient, and none had HPV6. HPV16 was found in 47% of HSIL; however, no case of HSIL had HPV18 or 45. HPV35 and HPV58 were the next most common types in HSIL, each occurring in 20% of cases, followed by HPV31 in 13.3%. Women <25 years had the highest HPV prevalence (30%), with a drop to less than 10% in those >44 years. HPV18 was twice as prevalent as HPV16 in women <25 years and demonstrated a steady decline with increasing age.

Conclusions: Although women <25 years old were underrepresented, these data suggest that the cervical HPV profile of Belizean women differs somewhat from that of women in the rest of Central America. These data are important with regard to the development of HPV vaccines that might be used in this region.

363 Grading Follicular Lymphomas/Diffuse Large B Cell Lymphoma by Fine-Needle Aspiration Cytomorphology and Flow Cytometry

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Background: Fine-needle aspiration (FNA) is useful for the diagnosis and classification of lymphoproliferative disorders. The diagnosis of follicular lymphoma (FL) and its distinction from diffuse large B cell lymphoma (DLBCL), however, require grading the neoplasm for optimal clinical management. There is no widely accepted means of grading based on FNA sampling alone, although a cutoff of >40% large cells has been suggested for classification as DLBCL. The purpose of this study was to determine if accurate grading of FL/DLBCL can be accomplished by cytomorphology or flow cytometry (FC).

Design: We studied the cytomorphology and flow cytometry data derived from 29 FNA specimens and the histology and flow cytometry data for 63 histologic specimens of FL/DLBCL; an additional 28 histologic specimens were studied as follow-up to the FNAs. A grade was assigned to the histologic samples by consensus according to the WHO scheme; WHO grades were then lumped into two clinically significant grades: "low grade" (WHO grades 1 and 2) and "high grade" (WHO grade 3 and DLBCL). For each FNA sample, we quantified the percentage of large cells by cytomorphology, assigning the specimen "high grade" if large cells comprised >40% of the cellularity. For all the lymphoma samples, we calculated the mean forward scatter and side scatter for the neoplastic cells by FC and attempted to establish cutoffs corresponding to histologic grade.

Results: Concordance on the grade assigned to the FNA sample and follow-up histologic specimen was 79% (23/29). For the FC cutoffs chosen (forward scatter > 1.2 or side scatter > 2.0, both normalized with accompanying T cells as an internal standard), the concordance between the grade assigned by FC and histology was 78% (72/92). The concordance on histologic grade among 3 pathologists (2 reviewers and the original pathologist who interpreted the biopsy) was 85% (77/91).

Conclusions: FL/DLBCL can be graded with good reliability by an evaluation of the percentage of large cells on FNA samples and by simple cutoffs based on forward/side scatter as determined by FC.

364 HPV Genotyping of ThinPrep (Cytec) Cervical Cytology Samples with Weakly Positive Hybrid Capture 2 (Digene) Results

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Background: Reflex testing for high risk (HR) human papillomavirus (HPV) on ThinPrep Pap (Cytyc) samples with diagnoses of ASCUS is the recommended standard. The Hybrid Capture 2 (HC2) assay (Digene) is currently the only FDA-approved method for this testing, although PCR-based research genotyping kits including the Linear Array (LA) HPV Test (Roche) are available. The significance of low positive (below 10 RLU) HC2 values is unknown, and Digene recommends repeat testing of values < 2 RLU. This study compares low positive HC2 values with genotyping results using the LA kit.

Design: 100 ThinPrep samples from 2007-2008 with HC2 results between 0.1 and 7.0 RLU were repeated and tested with the LA kit for both HR and low risk (LR) HPV types. All cytology slides were reviewed to re-evaluate the rendered diagnosis, and any additional follow-up cytology or histology was recorded if available.

Results: 49% of indeterminate (RLU 0.1-7.0) cases were positive for different HR genotypes, compared to 4.4% positives with repeat HC2. 21% of the cases were positive for HPV 16 and/or 18, but many other HR genotypes including 33, 39, 52, 56, 66, and 68 were identified. Of the samples indeterminate for HR HPV by HC2 that had only LR genotypes by LA, 21% contained HPV 53. This was twice the frequency of any other LR genotype.

Conclusions: ASCUS cases followed by positive HR HPV testing carry clinical implications, including referral for colposcopy. We believe that repeat testing of samples with low positive HC2 results has the potential for under-calling results and that screening with an alternate test such as the LA genotyping kit may be more sensitive. The high frequency of finding HPV 53 in cases of low positive HC2 HR HPV results may indicate a LR HPV type causing cross-reactivity in the HC2 assay.

365 Correlation of Aspiration Cytology of Ovarian Cystic Masses with Histology

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Background: There is conflicting evidence regarding the diagnostic accuracy of cytological evaluation of ovarian cystic lesions with sensitivities ranging from 25-76% and specificities from 97-100%. The purpose of this study was to evaluate the accuracy of aspiration cytology of ovarian cystic masses and to identify pertinent clinical and radiologic parameters of both benign and malignant lesions.

Design: Following IRB approval, 67 cases were identified to have FNA of an ovarian cystic mass followed by a cystectomy/oophorectomy. All cases were performed and evaluated at Fletcher Allen Health Care from 2000-2007. Radiographic and clinical data were obtained from chart review. Histologic diagnosis was used as the definitive standard upon which correlation with the five parameters of cytologic diagnosis, radiographic size (>5cm or >10cm), architectural complexity, and serum CA125 level (>35 U/ml) was made. Sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) were calculated. Chi square (x^2) analyses for the tests of significance and independence were calculated.

Results: Ten of the 67 cases were malignant, including 2 metastatic neoplasms and 5 borderline tumors. Of the 10 malignant cases, 5 were atypical/malignant and 3 non-diagnostic/paucicellular on cytologic assessment. The five parameters were independent of one another and correlation with malignancy was found not to be significant.

	Sensitivity	Specificity	PPV	NPV	*x^2	*p
CA125 >35 U/ml	70%	85%	54%	92%	0.25	>0.05
Size >5 cm	90%	35%	21%	95%	0.039	>0.05
Size >10 cm	80%	77%	40%	95%	0.2	>0.05
Architecture-complex	86%	52%	21%	96%	0.066	>0.05
Cytology -atypical/malignant	50%	100%	100%	92%	0.46	>0.05

*if $X^2_{0.95}(1) \geq 3.84$, then significant association and $p<0.05$

Conclusions: Reasons for the low sensitivity (50%) of cytology were the paucicellular nature of aspirate (n=3), focality of ovarian borderline tumors (n=5), and surface involvement by metastatic cancer (n=2). If only primary ovarian malignancies were evaluated, (n=8), the sensitivity of cytology would have been 63%. Cytology in this series, however, was the only parameter with 100% specificity and 100% PPV. As there may be a trend for the use of neoadjuvant chemotherapy in the treatment of ovarian malignancy, it is imperative to evaluate the accuracy and reliability of FNA of the ovary.

366 PCR-Based Cervical Cytology HPV Screening Tests Require High Analytical Sensitivity To Ensure the Inclusion of All Patients with CIN3 among Test Positives

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Background: The relationship of cervical cytology sample HPV viral load to a patient's subsequent histopathological diagnosis is contentious. Additionally, there is uncertainty about the sensitivity required of PCR-based cytological HPV screening tests to ensure the inclusion of all patients with CIN3 among test positives. It has been suggested that a high 'analytical' sensitivity will result in the detection of patients with clinically irrelevant amounts of HPV and that PCR assays should be optimized in terms of 'clinical' sensitivity. This study has examined HPV16 viral load in relation to lesion grade and has investigated what represents a clinically relevant amount of HPV16.

Design: A novel quantitative (q) PCR assay for the HPV16 *L1* gene was developed using Plexor™ technology (Promega Corp) and optimized for the consistent detection of ≤10 copies of HPV16 in a high background of human sequences. The assay was applied to DNA extracts (50ng) from 126 (13 ASC-US, 31 ASC-H, 12 LSIL, 64 HSIL, 6 NILM) known HPV16 positive routine cervical scrape samples. Histological follow-up data (39 benign changes, 5 CIN1, 26 CIN2, 49 CIN3, and 1 SCC) were available for all 121 patients with abnormal cytology. For each sample, HPV16 viral load data were normalized as ratios of qPCR data for the GAPDH housekeeping gene.

Results: Normalized viral loads ranged from 5.3×10^{-5} to 3.1×10^{3} copies of HPV16 per cell equivalent (mean 70.7, median 1.6, SD 337.5). Viral load did not distinguish different grades of abnormal cytology from each other [p=0.58]; NILM sample HPV16 viral load was significantly lower than in abnormal samples [p=0.0001]. Viral load did not predict biopsy histological grade; there was no significant difference in the amounts of HPV16 preceding a diagnosis of benign changes, CIN1, CIN2, or CIN3 [p=0.75]. Regarding CIN3, preceding viral loads ranged from 4.6×10^{-4} to 3.1×10^{3} HPV16 copies per cell (mean 86.4, median 1.7, SD 448.2), or, 16.0×10^{0} to 52.5×10^{6} HPV16 copies per 50ng DNA sample; CIN3 viral load was significantly higher in patients aged 16-25 years of age (n=21) than in patients aged 26-35 (n=22) [p=0.01].

Conclusions: These data show that HPV viral load testing is a poor predictor of histopathological grade and suggest that PCR-based HPV screening tests (currently under FDA-review) require application at high analytical sensitivity to avoid false negative HPV tests from potential CIN3 patients.

367 Fine-Needle Aspiration of Follicular Patterned Lesions of the Thyroid: Diagnosis, Management and Follow-Up According to National Cancer Institute Recommendations

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Background: The National Cancer Institute (NCI) State of the Science Conference on thyroid fine-needle aspiration (FNA) proposed that follicular patterned lesions can be divided into two diagnostic categories; follicular lesion of undetermined significance/rule-out follicular neoplasm (FLUS/ROFN) and follicular neoplasm/suspicious for follicular neoplasm (FN/SFN). The former group can benefit from repeat FNA (RFNA) to arrive at a definite diagnosis and the latter should undergo surgical excision for histologic characterization (follicular adenoma vs. carcinoma). In this study we report the combined experience from our institutions with thyroid FNA cases that can be placed into NCI designated thyroid FNA diagnostic categories for follicular patterned lesions.

Design: The case cohort comprised of 598 cases in 436 females and 162 males (average age 55 years); as proposed by NCI 327 cases could be classified as FLUS/ROFN (diagnosis based upon presence of one or combination of the following: increased cellularity, nuclear atypia and microfollicles) and 271 as FN/SFN (diagnosis based upon presence of one or combination of the following cytologic features: monotonous cell population, microfollicles). Surgical pathology follow-up was available in 142/327 (43%) cases diagnosed as FLUS/ROFN and 169/251 (67%) as FNA/SFN.

Results: 150/327 (46%) cases classified as FLUS/ROFN underwent RFNA. The RFNA diagnoses were 101 cases benign, 13 FLUS/ROFN, 27 FNA/SFN, 7 suspicious for papillary carcinoma and 2 as papillary thyroid carcinoma. The malignancy rate on surgical excision in the RFNA group was 35% as compared to 19% without RFNA. The malignancy rate on surgical excision in cases diagnosed as FN/SFN was 21%.

Conclusions: The management of thyroid lesions diagnosed as FLUS/ROFN by RFNA is an effective way to triage patients since the malignancy rates are different in cases with or without RFNA (35% vs. 19%). The malignancy rate (21%) of cases diagnosed as FN/SPN is similar to what has been reported by other authors.

368 The Grading of Small Biopsy Samples of Ovarian Carcinoma: Relationship to Response to Neoadjuvant Chemotherapy

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Background: Neoadjuvant chemotherapy may be employed for advanced-stage ovarian carcinomas unlikely to achieve optimal debulking at primary surgery. Initial chemotherapy with interval debulking increases the likelihood of optimal cytoreduction which is a significant prognostic feature. However, systematic grading of primary surgical specimens has demonstrated that low-grade tumors are more resistant to platinum-based therapy. The purpose of this study was to uniformly grade diagnostic biopsies of women with advanced stage ovarian cancer destined for neoadjuvant chemotherapy with interval debulking for correlation to clinical outcome.

Design: All women with ovarian carcinoma treated surgically at our institution between the years of 1995 and 2003 were retrospectively reviewed to identify those who were given neoadjuvant chemotherapy. All diagnostic material that preceded the initiation of chemotherapy were reviewed and scored by three independent cytopathologists using an adapted version of the Shimizu-Silverberg system to account for the predominance of cytology samples. Specifically, single cell pattern was interpreted as an architectural score of 3, and mitotic count was scored as 1 for zero, 2 for rare (1-2/100 cells) or 3 for numerous (>2/100 cells) mitotic figures. The scores were correlated to cytoreduction status and overall clinical outcome data.

Results: Of 200 women treated for ovarian carcinoma, 98 (49%) received primary chemotherapy, and 72 had archived slides available for review. The consensus review designated 10 grade 1, 36 grade 2, and 26 grade 3 carcinomas. Grade 1 tumors tended to have suboptimal interval debulking (3 of 10) when compared to grade 2/3 tumors (6 of 62) (p=.07). However, grade 1 tumors had a longer average post-surgical remission period of 569 days, compared to 329 days for grade 2/3 tumors (p=.045). There was no significant difference in the time to death (ave, 952 days); however, 30% of patients with grade 1 tumors had died of disease, compared to 53% of those with grade 2/3 tumors at last follow up (ave follow up, 1115 days).

Conclusions: Uniformly applying a grading system to small tissue biopsies and fluid samples for presumed high-stage ovarian carcinoma can identify low-grade lesions that have a tendency for suboptimal debulking after neoadjuvant chemotherapy. Longer remission periods after surgery may reflect slower growing tumors with fewer deaths due to disease.

369 HPV Genotyping Is Useful in Stratification of Women with Low Grade Squamous Intraepithelial Lesion (LSIL) on Cervical Pap Smears

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Background: Women with LSIL on Pap smears are all referred for colposcopy however underlying cervical intraepithelial neoplasia (CIN) 2/3 is identified in only 10-15% cases. Triage with HR HPV testing is not feasible due to the high rate of positivity. We have recently shown that women with ASCUS cytology may be stratified for aggressive management based on HPV 16 testing. This study was undertaken to determine if HPV genotyping could further stratify women with LSIL for personalized management.

Design: Residual SurePath samples from 52 Pap smears with a cytologic diagnosis of LSIL underwent automated DNA extraction and HRHPV genotyping using the COMPLeTe Care HPV assay (Physicians Reference Laboratory, Overland Park, Kansas), a multiplex PCR assay which simultaneously detects and types all of the 15 HR HPV (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 68, 73, 82) with appropriate positive and negative controls and beta globin as an internal control. The test has an analytic sensitivity of <10 copies of HPV/reaction, that corresponds to <125 copies/ml of Pap samples. Results were correlated with follow up diagnosis (Table).

Results: 36 (69%) of the samples were HR HPV positive with 21 (40%) showing multiple infections containing 2 to 4 HPV types. HPV16 was detected in 7 (13%), and HPV18 in 4 (8%) samples. The most common HPV types were 56 and 59 (11 samples, 21% and 10 samples, 19%) The age of patients varied from 18-73 y (mean 34.6y, median 27y). Follow up information (biopsy/repeat Pap smears) was available in 33 (91%) of the women with HR HPV.

	Follow UP Diagnosis		
LSIL plus	Negative	CIN 1	CIN 2/3
HPV16+ (5)	0 (0%)	2 (40%)	3 (60%)
HPV18+ (4)	2 (50%)	2 (50%)	0 (0%)
HRHPV non16/18+ (24)	8 (33%)	12 (50%)	4 (17%)*

* LSIL samples contained HPV types 45,51,52,56,58,59

Conclusions: HPV genotyping is useful in stratifying women with LSIL into two categories: those positive for HPV 16 with 60% risk and those positive for Non16 HPV types with 14% risk of CIN2/3 (p=0.05). Additional studies are warranted to determine individual risks for the Non 16 HPV viruses.

370 Significance of Type-Specific HPV Persistence in Women over 30 with Cytologic Diagnoses of NILM or ASC-US

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Background: HPV DNA testing is widely used in the triage of women with Pap ASC-US as well as more recently as an added screening test in women over 30 with Pap tests diagnosed as NILM. Persistence of HPV infection has been identified as one of the most important factors in the progression of HPV infection to significant cervical lesions. We have reviewed our experience with HPV testing and typing of women with ASC-US and NILM to examine the significance of type-specific persistence of HPV in this population.

Design: We identified all cases with an initial diagnosis of either ASC-US or NILM that had HPV determination and typing performed by PCR on the residual Surepath sample from 2002 to 2007. Women over 30 with repeat HPV determinations within 24 months after the initial HPV determination were identified and the results of the HPV test, follow-up Pap tests and biopsies within 6 months were entered into a spreadsheet and analyzed statistically using SPSS 12.0.

Results: A total of 1,053 patients with repeated HPV DNA test were included in the analysis. The corresponding initial Pap test was diagnosed as ASC-US in 796 and as NILM in 167 cases. The mean ages was 51.67 (min:39; Max:95). Six hundred and seventy three patients were included were negative for HPV both in the initial and in the follow-up HPV determination (63.9%), 169 (16%) had transient HPV infections (were positivity in only one of the HPV determinations), and 130 (12.3%) were repeatedly positive for the same HPV type, while 81 (7.7%) were positive for HPV on two or more determinations but had different HPV types. The mean time between the tests was 17 months. Patients with repeated positivity for HPV with the same or different HPV type were 30 times more likely to have ≥LSIL on follow-up than womenb who where negative for HPV (p<0.0001, OR= 30.67, 95 %CI = 11.820 -79.049). Patients with high risk persistent HPV infections, but having different types of high risk HPV were 23% more likely to have follow-up of ≥LSIL than patients with transient high risk HPV infections. (p=0.009, OR=1.23, 95%CI 1.074 –1. 410). However, patients

with high risk persistant HPV infection with the same type of HPV were 10 times more likely to have a follow-up result ≥LSIL than patients with transient high risk. (p< 0.0001, 95%CI 1.558 – 64. 198).

Conclusions: Repeated HPV testing with a method allowing high risk HPV typing may be useful in the evaluation of women with repeated HPV tests as part of ASC-US triage or co-screening with Pap and HPV tests.

371 Adenocarcinoma in Papanicolaou Specimens from Patients with Endometrial Carcinoma: Correlation with Tumor Stage and Endocervical Involvement

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Background: Atypical glandular cells can be found in Papanicolaou specimens from patients with endometrial carcinomas (EC). The extent of surgery in endometrial adenocarcinomas depends on the stage of the endometrial carcinoma. The stage can sometimes only be determined intraoperatively and the accuracy of this evaluation is variable. We propose to evaluate the correlation between the presence and number of atypical glandular cells in Papanicolaou smears with stage. The findings might be helpful in determining surgical management. We also proposed to evaluate the same criteria to determine if there was any correlation with endocervical involvement.

Design: We reviewed our database to identify cases of ECs where the preoperative Papanicolaou specimens showed the presence of atypical glandular cells. The specimens were evaluated to confirm the presence of atypical cells similar to the ones seen in the tissue sections. The number of cells was recorded and correlated with the tumor stage and endocervical involvement by EC.

Results: A total of 36 cases of EC met the above criteria. The relationship between tumor stage and number of atypical cells in the Papanicolaou specimen is shown is Table 1. More than 10 atypical cells in Papanicolaou specimens is noted in 32% of the stage I EC, while 50% or more cases of stage II-IV also showed increased number of atypical glandular cells.

Table 1

Stage/number of cells	1-10 cells	>10 cells
I	17	8
II	0	3
III	4	4
IV	1	2

The correlation between endocervical involvement and number of atypical cells in the Papanicolaou specimen is summarized in table 2. More than 10 atypical cells was noted in 37% of cases without endocervical involvement by EC and in 78% of cases with endocervical involvement by EC.

Table 2

Endocervical involvement/number of cells	1-10 cells	>10 cells
No	19	11
Yes	2	7

Conclusions: Although the Papanicolaou specimens are not a sensitive screening test for EC, the presence of increased number of atypical glandular cells in Papanicolaou specimens from patients with EC is associated with higher tumor stage and endocervical involvement.

372 Impact of Chromosome 17 Polysomy and Equivocal Immunostaining Results on the HER2 Status Determined by Chromogenic In Situ Hybridization: A Study of Two Institutions Using the New ASCO/CAP Scoring Criteria

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Background: The new ASCO/CAP guidelines mandated that a new HER2 assay should show ≥95% concordance with another validated assay for positive and negative results before the test is offered. Because CISH defines HER2 status usually based on an absolute HER2 gene copy number, without chromosome 17 corrections, the reliability of CISH on the cases with chromosome 17 polysomy and equivocal IHC is of interest.

Design: Paraffin tissues of 286 breast carcinomas were tested for HER2 status by FISH (Vysis) and IHC (HercepTest) at MD Anderson (site A) and University of Tampere (site B). Cases showing chromosome 17 polysomy or equivocal IHC score were selected to examine the concordance between CISH and FISH at each site and the reproducibility of CISH between two sites. Results were interpreted by pathologists at each site using the new ASCO/CAP scoring criteria.

Results: Polysomy of chromosome 17 was found in 51 cases at site A and 22 cases at site B. The total number of tumors showing polysomy 17 at either or both sites was 66. Using the three-category criterion (amplified, equivocal and non-amplified), the concordance between the two methods was 91.7% at site A and 95.5% at site B, and the intersite agreement on CISH was 90.3%. Using the two-category criterion (i.e., excluding equivocal cases), the concordance between the two methods was 100% at both sites, and the intersite agreement on CISH was 100%. With HercepTest, 36 cases showed equivocal score at site A and 43 cases at site B, 49 cases had equivocal score at either or both sites. Using the three-category criterion, the concordance between the two methods was 85.3% at site A and 87.8% at site B, and the intersite agreement on CISH was 86.7%. Using the two-category criterion, the concordance between the two methods was 96.7% at site A and 97.3% at site B, and the intersite agreement on CISH was 97.4%.

Conclusions: The concordance between CISH and FISH on cases with chromosome 17 polysomy and with equivocal IHC score achieves the high concordance mandated by the ASCO/CAP guidelines, with very high reproducibility of CISH between two sites.

373 Is the Immediate Evaluation of Touch Imprint Cytology from CT-Guided Core Needle Biopsies of Mass Lesions Helpful?

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Background: Computed Tomography guided core needle biopsy (CTCNB) is a minimally invasive, safe and effective manner of tissue sampling in many organs. Some institutions employ pathologists in the immediate evaluation of CTCNB using touch imprint cytology (TIC) to guide the radiologist in determining whether the biopsy needle is in the correct location and to ensure adequate tissue sampling. The aim of our study is to determine the impact of on-site evaluation of TIC to minimize the number of passes required to obtain adequate tissue for diagnosis.

Design: A retrospective review of all CTCNBs from 2004 to 2008, where a pathologist was present for on-site TIC evaluation was performed. The cases included CTCNBs from masses in various organs. Each case was evaluated for the number of passes required before TIC was interpreted as adequate for diagnosis.

Results: A total of 140 CTCNBs were included in the study (liver, lung, kidney, sacral, paraspinal, omental, splenic and adrenal masses). Of the 140 cases, 109 were diagnosed as malignant and 28 as benign. In 106 cases (75.7%), the biopsies were determined adequate by TIC on the first pass, 19 (13%) on the second pass and 7 cases (5%) on the third pass. Only in 5 cases (3.6%), more than 3 passes were required before diagnostic material was obtained. Three cases (2.14%) were interpreted as inadequate both on TIC and on the final diagnosis. Of the biopsies deemed adequate on the first pass, 71% resulted in either termination of the procedure, or only one additional pass was obtained. In 5 cases, based on the TIC evaluation, a portion of the sample was sent for either flow cytometric analysis or cytogenetics.

Conclusions: In the majority of cases, adequate material was obtained in the first pass of CTCNB, and once this was obtained, either no additional passes, or one additional pass was performed. This study demonstrates the utility of on-site evaluation in minimizing the number of passes required to obtain a sufficient amount of diagnostic tissue and for specimen triage for ancillary studies, which in turn decreases the risk to the patient and decreases costs.

374 Polyomavirus Remains a Diagnostic Pitfall in Urinary Tract Cytology

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Background: Cells showing polyoma virocytopathic changes and high-grade urothelial carcinoma cells may closely mimic one another, to the point of being virtually indistinguishable. This presents a diagnostic dilemma, as these diagnoses have markedly different implications. Our study retrospectively evaluates our efficacy in distinguishing polyomavirus infection from urothelial carcinoma, and attempts to characterize their respective cytomorphologies.

Design: A departmental database search was conducted to identify patients with urine cytology specimens given a diagnosis of polyomavirus infection between January 2005 and July 2008. Of these cases, those that were either subsequently or previously diagnosed with urothelial carcinoma were included in our study. A separate search for patients diagnosed cytologically with polyomavirus who had at least two years of negative follow-up was conducted to establish a control group. The slides of these specimens were reviewed by two board certified pathologists, and widely-published features of polyomavirus infection and urothielial carcinoma were semiquantitatively analyzed.

Results: Our search identified fourteen lower urinary tract cytology specimens with features previously reported as polyomavirus from patients in our study group. Ten additional patients diagnosed with polyomavirus, without any history of carcinoma, were included in the control group. The slides from these cases were reviewed while blinded to the previous diagnoses. The differences between the two groups were not statistically significant for any of the major attributes, with the exception of nuclear membrane irregularity. More traditional identifying features of polyomavirus, such as the presence of nuclear inclusions, "comet cell" morphology, nuclear membrane thickening, and lace-like chromatin pattern, showed no statistical differences between the two groups.

Conclusions: We found statistically significant overlap in the morphologies of polyomavirus and urothelial carcinoma. Most notably, the "glassy" nuclear inclusions seen with polyoma appear to be much less specific than is generally appreciated. However, the presence of nuclear irregularity showed statistically significant predictive value. In many cases, the cytomorphologies of polyoma virus infection and urothelial carcinoma have enough common characteristics that even rigorous use of published morphologic criteria may be insufficient to reliably distinguish between the two.

375 Polyclonal S100A1 Antibody Distinguishes Oncocytoma from Chromophobe RCC in Cytogenetically-Proven Renal Tumors and Fine Needle Aspirations

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Background: Recent reports have shown that a monoclonal antibody (Ab) to S100A1 distinguishes renal oncocytic neoplasms with relatively high sensitivity and specificity. However, the Ab used is no longer commercially available, and the reported cases were not correlated/confirmed with cytogenetics. The aims of this study were 1) to re-establish the sensitivity and specificity of a commercially available polyclonal S100A1 Ab in cytogenetically-proven renal epithelial tumors, and 2) to determine if S100A1 is useful in distinguishing oncocytic neoplasms evaluated by FNA.

Design: A polyclonal Ab to S100A1 was used to stain 1) 2 tissue microarrays (TMAs) containing 171 consecutive renal tumors with karyotypes (125 clear cell RCCs, 25 papillary RCCs, 10 chromophobe RCCs, and 11 oncocytomas); 2) sections of cytogenetically proven oncocytomas (12) and chromophobe RCCs (6) from nephrectomy

specimens; and 3) 25 renal oncocytic neoplasms obtained by FNA. All cases were also stained for conventional S100B protein.

Results: In the TMAs, S100A1 was positive in 67% of clear cell RCCs, 60% of papillary RCCs, and 82% of oncocytomas; all chromophobe RCCs were negative. In the cytogenetically-proven nephrectomy tumors, all oncocytomas were positive for S100A1 and all chromophobe RCC were negative. In cell block (CB) preparations, it was necessary to confirm the presence of diagnostic tissue by H&E, as material was often scant and/or admixed with non-neoplastic renal tubules which are also positive for S100A1. Of the 25 CBs, S100A1 was positive in 18/19 (95%) oncocytomas (cytoplasmic pattern) and equivocal in 1 case; 4/6 chromophobe RCCs were negative for S100A1, 1 was positive (membranous pattern), and 1 was equivocal. All TMA, whole mount and FNA cases were negative for conventional S100B protein.

Conclusions: Consistent with prior reports, S100A1 demonstrates low overall specificity for oncocytoma, as clear cell and papillary RCC are also positive. However, when the differential diagnosis is oncocytoma vs chromophobe RCC, sensitivity and specificity are very high (100% in cytogenetically-proven cases). Focal staining in TMA and CB preparations decreases sensitivity and specificity (90% and 93%, respectively; combining TMA and CB specimens) due in part to sampling error; however, a granular cytoplasmic pattern favors oncocytoma. Positive staining of admixed renal tubular epithelium is a potential pitfall when interpreting S100A1 in CBs. Conventional S100B Ab cannot be used for this differential diagnosis.

376 Clinical Evaluation of ProExC on Liquid-Based Cervical Cytology Specimens and Its Correlation with p16^{INK4a} and HC2 High-Risk Human Papillomavirus (hrHPV) Testing

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Background: The 2006 ASCCP consensus management guidelines recommend reflex hrHPV DNA testing for equivocal cytology (ASC-US) to increase the efficiency of cervical cancer screening by better risk stratification. This study was performed to evaluate a novel antibody cocktail (ProExC) in detecting hrHPV+ ASC-US cases and to compare its clinical usefulness with p16^{INK4a} and HPV testing.

Design: Liquid-based cervical cytology (CC) specimens were collected using BD SurePath method and routine Pap staining was performed. The ASC-US cases reported over the past one month were triaged for hrHPV testing. Residual samples were used to prepare two additional slides for ProExC and p16^{INK4a} immunocytochemical analysis. 16 additional CC samples received only for hrHPV DNA test were also included in this study. The slides were evaluated for presence of nuclear staining within cytologically abnormal epithelial cells. The sensitivity, specificity, PPV and NPV were calculated.

Results: The 52 slides stained with ProExC and p16 immunocytochemical stain (34 ASC-US, 13 NILM, 2 LSIL, 1 HSIL, positive and negative controls) were interpreted independently, and with no previous knowledge of the cytological diagnosis, by a pathologist[1] and a cytotechnologist[2], with 100% correlation to the reference results. Of these 50 cases, 14 cases were positive for ProExC, 9 for hrHPV and none for p16^{INK4a}. 3/34 ASC-US and 2/13 NILM cases showed false positive staining with ProExC and were negative for hrHPV DNA. This false positive staining was due to positively stained reactive endocervical cells.

Table 1: Correlation between cytological diagnosis and hrHPV, ProExC and p16INK4a results.

	No. of cases	hrHPV+ cases	ProExC+ cases	p16INK4a+ cases
ASC-US	34	6	9	0
NILM	13	1	3	0
LSIL	2	1	1	0
HSIL	1	1	1	0
	50	9	14	0

Table 2: Correlation between hrHPV and ProExC results.

	hrHPV+ cases	hrHPV- cases	Total no. of cases
ProExC+ cases	7	7	14
ProExC- cases	2	34	36
Total no. of cases	9	41	50

The sensitivity and specificity of the ProExC test was 78% and 83%, and the PPV and NPV was 50% and 94% respectively.

Conclusions: Using hrHPV testing as a bench mark, a negative ProExC test result almost excludes the presence of hrHPV infection. Thus, ProExC is a useful and a better ancillary diagnostic test than p16^{INK4a} in detecting hrHPV+ ASC-US cases and thus is helpful in identifying those cervical lesions that are most likely to progress.

377 Cell Chip Platform Using Nanomesh and Collagen Coated-PET Discs in Cervicovaginal Cytology

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Background: Adhesion of cells on surface is an important factor in cytology smear. According to development of nanotechnology, biocompatible nanofibers have been studied and many biomedical applications have been widely reported. Poly (3-hydroxybutyrte-co-3-hydroxyvalerate) (PHBV) is one of the most promising materials for tissue engineering. Composite solution of PHBV and the natural calf collagen peptide (PHCP), dissolved in 2 wt% 2,2,2-trifluroethanol (TTF), were electrospun on polyethylene teraphthalate (PET) film (nanomesh plate). Nanomesh plates and type I collagen-coated PET discs attached to a 10-holed slide (cell chip frame), using for high throughput screening and immune- and special staining of cervicovaginal cytology.

Design: We used remnant cervicovaginal cells in ThinPrep solution (Cytyc, MA, USA). After centrifuged and mixed with LiquiPrep (LGM international, FL, USA) solution, the cells were smeared on the representative cell chip. Among 19 epithelial abnormal specimens above ASCUS, 8 NILM specimens for negative control were arrayed in cell chips. Each specimen was smeared on nanomesh, collagen coated and conventional slide cell chips. Each cell chip had a HeLa cell line sample for positive control. Total 24 cell chips, including 6 nanomesh cell chips, 9 collagen coated slide cell chips and 9 conventional slide cell chips were made. The cell chips were respectively performed Papanicolaou stain, Ki-67 and p16 immunocytochemical stain. Namomesh cell chips were excluded in p16 immunochemical stain for problems in cell adhesion. Each of 10 samples were interpreted on one cell chip and compared with cytologic and final histologic diagnosis.

Results: Among total 57 tested disc, 21 samples were in agreement with conventional diagnosis, 20 samples showed category B and C disagreement, 16 samples were insufficient for diagnosis. Sensitivity of p16 for HSIL and squamous cell carcinoma was 66.6% and specificity was 81%. In Ki67, sensitivity was 58.3% and specificity was 51.7%.

Conclusions: Even though, cell chip had some defects, such as cellular overlapping and air bubbles on nucleus according to technologist, relationship between p16 and epithelial abnormality of cervical cytology, reported in literatures was well reproved in cell chip platform. The current results indicate that cell chip prepared from residual Thinprep material should be an economic, and high throughput adjuvant test platform with high reproducibility of cervicovaginal samples.

378 Determination of Oropharyngeal or Nasopharyngeal Squamous Cell Carcinoma Primary Site from Fine Needle Aspiration of Cervical Lymph Node Metastases

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Background: Fine needle aspiration of head and neck tumors often involves evaluation of enlarged cervical lymph nodes. In patients with metastatic squamous cell carcinoma (SCC) in these lymph nodes, the site of origin may not be clinically evident. The distinction between oropharyngeal and nasopharyngeal primary SCC has important management consequences. A reliable method of distinction would be of clinical benefit. Previous studies have documented the utilization of human papilloma virus (HPV) detection by in-situ hybridization (ISH) and surrogate markers for HPV (p16INK4a) for oropharyngeal SCC. In the current study, we evaluate metastatic SCC for HPV type 16, 18, 31, 33, 51 (by ISH), p16 and ProExC (surrogate HPV markers), and EBER reported in nasopharyngeal SCC.

Design: Forty patients between 2004 and 2008, with adequate cell block material of cervical lymph node metastatic SCC, were identified. ISH for high risk HPV (types 16,18, 31, 33,51) and EBV (EBER), and immunohistochemistry for p16 and ProExC were performed. The site of primary SCC was obtained by medical record review.

Results: Primary site was designated in 30 cases with 23 oropharyngeal, 2 nasopharyngeal, 5 other sites (anus, cervix, lung), and 10 unknown sites. High risk nuclear HPV was detected in 9 cases (22.5 %), nuclear and cytoplasmic overexpression of p16 in 15 cases (37.5 %), ProExC in 35 cases (87.5%), and EBER in 2 cases (5%). All cases with high risk HPV ISH also showed overexpression of p16; 6 (15%) showed p16 overexpression in the absence of HPV ISH. ProExC was positive in 19 cases (47.5 %) without co-existent expression of either p16 or HPV ISH. The sensitivity for HPV infection by both surrogate markers was 100%; specificity for p16 and ProExC was 77.4% and 16.1%, respectively. Seven (30%) oropharyngeal SCC were positive for HPV ISH and negative for EBV; one nasopharyngeal SCC (50%) was EBER positive and HPV negative.

Conclusions: HPV and EBER detection can serve as indicators for oropharyngeal and nasopharyngeal primary SCC respectively, however our data show that only a subset (30%) of oropharyngeal squamous cell cancers are high risk HPV related. Additionally, despite their high sensitivity for HPV infection, surrogate markers, especially ProExC, lack specificity.

379 Ex-Vivo Renal Fine Needle Aspiration in the Diagnosis of Renal Masses: A Prospective Controlled Study

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Background: Fine Needle Aspiration (FNA) of renal masses has been recognized as a potential tool for preoperative typing. However, retrospective studies, have been contradictory in diagnostic accuracy and applicability. The goal of the current study was to evaluate the accuracy and interobserver variability in a prospective and controlled fashion.

Design: Ex-vivo FNA was prospectively performed on 42 renal masses following nephrectomy obtained between 04/08 and 09/08. Using a 23-gauge needle, 5 passes were made, slides were stained with Papanicolaou (5) and Diff-Quik (5). The needles were rinsed in RPMI and CytoLyt for cytogenetic (CG) analysis and cell block preparation (CB). Two cytopathologists independently evaluated the aspirates to determine adequacy, cellularity, and diagnosis: malignant vs. benign, with tumor grading and subclassification. Concordance between the FNA interpretations, the final diagnosis and CG results were analyzed to determine the accuracy of FNA.

Results: All the FNAs performed, were adequate to render a diagnosis, although 16 were hypocellular. Fourty of 42 cases (95%) were accurately classified as benign/malignant. Three cases erroneously diagnosed as malignant were inflammatory myofibroblastic tumor (2) (both of which were hypocellular with rare highly atypical cells), and one benign mixed epithelial/stromal tumor (interpretive error). Combined the accuracy in subclassifying the tumors (40/42 cases) was 95%. Papillary RCC, leiomyosarcoma, and benign mixed epithelial and stromal tumor was subclassified inaccurately as RCC. CG evaluation showed that 12/14 cases (86%) are in agreement with the cytological/surgical diagnosis, and 2 cases did not have adequate material.

Accuracy of Diagnosis, Subclassification, Grading

	Pathologist 1	Pathologist 2	Combined
Benign vs Malignant	93%	93%	95%
Subclassification	81%	79%	95%
Grading	100%	98%	100%

Conclusions: In a setting where material can be obtained for CB as well as CG FNA has a high concordance with surgical diagnosis. It is particularly accurate in determining if a mass is malignant/benign and in grading neoplasms. Despite the lack of immunohistochemical analysis accuracy of subclassification was high which could further improve by cytopathologists collaboration. It is our belief that FNA has a valid role in the preoperative diagnosis of renal masses.

380 Comparison of hrHPV Prevalence in over 1300 Computer-Imaged Liquid-Based Pap Samples with and without TZ/ECS

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Background: Sampling of the transformation zone and endocervical cells (TZ/EC) has been widely regarded as a quality indicator in cervical program; however, the significance of a TZ/EC sample in promoting disease detection remains controversy. The purpose of this study was to determine whether or not there is an association between hrHPV DNA test results and TZ/EC sampling in over 1300 LSIL ThinPrep samples.

Design: A computer-based search of Copath files of MWH, UPMC was carried out over a 34 month period (July 2005-April 2008) to retrieve women with ThinPrep Pap Test (TPPT) reported as LSIL who also were tested for hrHPV. All TPPT were processed and prepared using an automated processor and imaged using the ThinPrep Imaging System. hrHPV DNA was detected by HC2. hrHPV detection rates were compared between women with either presence or absence of a TZ/EC sample. Statistical analyses were performed by Chi-square test or Fisher's exact test for small number.

Results: A total of 1351 LSIL TPPT also underwent hrHPV DNA testing. hrHPV positive rate was higher in young women. There was a trend of decline in the hrHPV DNA prevalence in older women groups. hrHPV detection rate was significantly higher in women <40 compared with women 40 years and older (85.6% vs. 70.0%, p<0.001). No statistically significant difference of hrHPV prevalence was present between women with and without a TZ/ECS except for age 20-29 year group in which hrHPV rate was slightly higher in TZ/ECS present group than that in TZ/ECS absent group.

Table 1 - Comparison of Age-Specific hrHPV Prevalence among Women with LSIL TPPT with and without TZ/ECS (10-Year Intervals)

	TZ/ECS Present			TZ/ECS Absent			
Age Group	**Tested No**	**Positive No**	**%**	**Tested No**	**Positive No**	**%**	**P Value**
10-	86	79	91.9	13	12	92.3	1.0*
20	382	342	89.5	74	60	81.1	0.049
30-	281	228	81.1	45	33	73.3	0.498
40	232	160	69.0	39	29	74.4	0.498
50-	103	69	67.0	39	27	69.2	0.799
60-	36	29	80.5	13	10	76.9	1.0*
≥ 70-	4	1	25.0	4	4	100.0	0.143*
Total	1124	908	80.8	227	175	77.1	0.185

*Fisher's exact test

Conclusions: This is the largest study to document hrHPV DNA detection rates in women with LSIL Pap tests with and without TZ/ECS. HC2 hrHPV DNA detection in LSIL TPPT vials is independent of cytologic sampling of TZ/ECS. hrHPV detection rate is lower in older women with LSIL Pap tests. Triage of older women with LSIL Pap test using hrHPV DNA testing might be helpful for these women's risk assessment.

381 Significance of Mitotic Activity in Post-Menopausal Atrophic Smears

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Background: Dysplasia can be difficult to discern in some atrophic gynecologic smears. Crowded sheets of cells with enlarged, hyperchromatic nuclei can be seen in both dysplastic lesions and atrophy. The presence of mitotic figures in dysplastic smears has been suggested as a discriminating feature. The aim of this study was to determine the value of mitotic activity in identifying dysplasia in post-menopausal atrophic smears.

Design: Liquid based Pap tests obtained from post-menopausal women from 2002 were examined for the presence of atrophy, syncytial cell groups and mitotic activity. Clinical history and pathology records, from 2002 – 2007, were reviewed for dysplasia or malignancy for those patients whose Pap test showed mitotic activity. Similar information was collected for a subset of 14 post-menopausal patients with a Pap test without mitotic activity (control group). The patient's HPV status, previous history of malignancy and use of hormonal therapy were also recorded.

Results: The average age for this group of patients was 64.6 years (range, 46-85 years). 206 Pap tests showed syncytial cell groups. Twelve of these Pap tests showed the presence of mitotic figures. HPV was negative in the 9 patients who were tested. There were seven patients with a history of breast cancer, one each with endometrial carcinoma and CIN 3, and three with no history of malignancy. Four of the 12 patients had received Tamoxifen and 1 patient was on hormone replacement therapy. None of the 12 patients have developed dysplasia or malignancy at the time of last follow-up. In the control group, HPV was positive in 1 out of the 4 patients who were tested. None of the control group patients received hormonal therapy and other follow-up was negative.

Conclusions: 1. Mitotic figures can be seen in post-menopausal atrophic smears in the absence of dysplasia. 2. Mitoses alone cannot be taken as evidence of dysplasia in atrophic smears. 3. Mitotic activity may be related to hormone therapy in at least a subset of patients.

382 Thyroid Ultrasound Guided Fine Needle Aspiration Biopsies (US-FNA) Performed by Cytopathologists. The Lexington KY. Veterans Administration Hospital Experience

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Background: Recently, the demand for thyroid US-FNA for both palpable and non-palpable masses has increased; this may limit cytopathologists from performing aspirations of this anatomic site. Our study shows that with adequate training a cytopathologist can efficiently and safely utilize ultrasound (US) as a localizing devise for thyroid aspirations.

Design: Cytologic smears of 100 consecutive patients that underwent thyroid US-FNA by a cytopathologist, were reviewed for adequacy, duration of procedure, number (#) of passes, # of slides, percentage (%) of surface area smeared per slide(p/s), # of follicular cell groups p/s, % of obscuring blood, diagnosis (dx), histologic correlation and immediate and remote complications. All patients had thyroid lesions ≥ 1cm diagnosed and documented by a radiologist with 9 to 21 months of follow up. Alcohol prep pads (70%) were used to cleanse the skin without sterile draping. Soap and water solution instead of US gel was utilized. The biopsy was performed using 27 G x 1 $^{1/2}$ needles with no aspiration and without local anesthesia. Pressure with sterile gauge was applied after each pass. Biopsy material was smeared using the Karolinska methodology and subsequently stained [Papanicolaou and Diff-Quik (DQ)]. Immediate evaluation to determine adequacy was performed after each pass using DQ stain.

Results: All patients had adequate material. On average, the duration of the procedure was 30 minutes, and each patient had 3 passes and 8 slides with a surface area of 40-50% p/s. Between 6 and 30 groups of follicular cells p/s were seen in 90 patients. Six patients had an average of 2 cell groups and 4 patients had an average of 5 cell groups p/s. Obscuring blood was seen in < 10 patients. Ninety six patients had a diagnosis consistent with colloid nodule/goiter and/or a component of lymphocytic thyroiditis. Two patients had histologically confirmed papillary carcinoma and another 2 patients had follicular lesions that proved to be minimally invasive follicular carcinoma and follicular adenoma. No acute or late complications were reported.

Conclusions: Using the described methodology, US-FNAB of thyroid lesions can be performed by a cytopathologist without complications and can be cost effective, efficient and diagnostically accurate.

383 An Investigation of Atypical Squamous Cells, Cannot Exclude HSIL (ASC-H) and Follow-Up Outcomes with Reflex HPV Testing

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Background: The recommended management of women with ASC-H is immediate colposcopic examination without HPV testing. Our objective was to correlate HPV results in ASC-H cases with findings at colposcopy.

Design: 264 cases of ASC-H were identified from a total of 94,603 PAP tests performed between January 2007 and August 2008. Testing for high risk HPV had been performed in 103 cases and 39% were positive. Clinical follow-up included either colposcopy (172 cases) or a second PAP test (25 cases). 29 cases were lost to follow-up in our system. Biopsy results were categorized as negative, CIN 1, or CIN 2+; PAP results were graded as NILM, LGSIL, or HGSIL.

Results: In the 28 HPV+ patients who underwent colposcopy, 14% had CIN2+ lesions, 57% had CIN1, and 29% had negative biopsies. Of the 37 HPV- patients, none had CIN2+ lesions, 38% had CIN1, and 67% were negative. Of 107 patients who underwent colposcopy without HPV testing, 11% had CIN2+ lesions, 33% had CIN1, and 56% had negative biopsies. Of 25 patients who were followed only by repeat PAP testing, 8% had HGSIL, 8% had LGSIL, and 84% had NILM on follow-up. The study cohort was divided into 3 age groups: 18-35, 36-55, and over 55. All 4 of the CIN2+ lesions in HPV tested women occurred in the HPV+ group, 2 each in the younger groups None of the HPV- women had CIN2+ lesions, but a substantial number (36-40%) had CIN1 lesions. The proportion of CIN1 lesions was much greater in the HPV+ youngest women (71%), than in the 36 to 55 year-old group (40%),(p = 0.01).

		HPV testing		Follow-up			
				Colposcopy			PAP test only (NILM, LGSIL, HGSIL)
	Cases	Number tested	% positive	HPV+ (neg/CIN1/CIN2+)	HPV- (neg/CIN1/CIN2+)	Colposcopy only (neg/CIN1/CIN2+)	
18-35 yr	104	46	56.5	17 (18%/71%/11%)	11 (64%/36%/0%)	50 (42%/42%/16%)	8 (75%/0%/25%)
36-55 yr	100	47	25.5	10 (40%/40%/20%)	21 (62%/38%/0%)	44 (74%/23%/5%)	9 (78%/22%/0%)
>55 yr	31	10	20.0	1 (100%/0%/0%)	5 (60%/40%/0%)	13 (54%/31%/15%)	8 (100%/0%/0%)
Total	235	103	38.8	28 (29%/57%/14%)	37 (62%/38%/0%)	107 (56%/33%/11%)	25 (84%/8%/8%)

Only 42% of the youngest women without HPV testing had negative biopsies compared to 73% of those 36 to 55 years old, and 55% of the oldest women. CIN2+ lesions were found in each age group (16%, 4.5%, and 15%, youngest to oldest, respectively). Although only 25 women with ASC-H were followed only by PAP smear, 84% of them had negative findings on subsequent testing.

Conclusions: The role of HPV testing in ASC-H is unclear. Biopsy of HPV+ cases is supported, because all CIN2+ cases occurred in that group. A negative HPV result is not associated with a high grade lesion; however, if these patients had not been biopsied, many CIN1 lesions would be missed.

384 HPV Results on Unsatisfactory Gynecologic Pap Specimens

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Background: Unsatisfactory pap smears are a common problem in gynecologic cytology. Although Human Papilloma Virus (HPV) testing is requested on these specimens, the validity of HPV results from unsatisfactory pap smears is not well studied.

Design: A search for all unsatisfactory pap smears (ThinPrep and SurePath) with concurrent HPV tests (Digene HybridCapture II) during a 1 year (2006) period was

performed utilizing the pathology database system at Stanford Hospitals & Clinics. The cause of the unsatisfactory specimen and the HPV results were recorded. The patients' pap smear and HPV test results for up to 1 year before and after the unsatisfactory specimen were noted. In addition, the HPV+ rate was determined for 1 year of NILM and abnormal paps.

Results: 166 unsatisfactory pap smears with HPV tests were found, with 11 positive (6.6%), 152 negative (91.5%) and 3 equivocal (1.8%) cases. 27 cases had HPV testing within one year of the unsatisfactory specimen. 4/27 cases had discordant results: 1) equivocal to positive (106 days), 2) negative to equivocal (115 days), 3) positive to equivocal (365 days), and 4) negative to positive (30 days). All four cases were unsatisfactory due to scant cellularity, one of which also had obscuring lubricant. There were also 35 pap tests within 1 year of the original specimen: 34 NILM following a negative HPV, and 1 LSIL following a positive HPV. Overall, there was 1 definite false negative HPV result out of the 62 cases (1.6%) with prior or subsequent HPV or pap tests. The false negative case was negative due to scant cellularity. The HPV positive rate for unsatisfactory specimens was compared to baseline HPV rates by diagnosis, showing similarity to the NILM HPV rate.

HPV+ Rates

DIAGNOSIS	TOTAL # CASES	HPV+ (%)
UNSATISFACTORY	166	6.6
NILM	200	6.0
ASC-US	525	57
ASC-H	63	73
LSIL	100	82
HSIL	14	86
REASON FOR UNSATISFACTORY:		
Scant cellularity	130	6.2
Obscuring lubricant	24	8.3
Inflammation	3	0
Insufficient squamous epithelial component	5	20
Blood	3	0
Acellular	1	0

Conclusions: There are few studies in the literature examining whether unsatisfactory pap smears provide enough material for a valid HPV test result. This study provides preliminary evidence that these specimens provide reliable HPV results with a low false negative rate.

385 Cervical Cytology Diagnosis by Infrared Micro-Spectral Imaging

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Background: Infrared micro-spectral imaging (IRMSI) is a novel approach for cytology diagnosis. This optical technique detects subtle biochemical changes within normal and dysplastic cells of different types and differentiation. Computer based algorithms analyze spectral differences within individual cells and provide an objective and reproducible tool in diagnosis. These differences may be seen before morphological changes are apparent, providing additional sensitivity for early stage abnormalities. We have studied the correlation between spectral and conventional cytology of exfoliated squamous cervical cells.

Design: Cervical samples (>100) were preserved in SurePath solution, centrifuged (CytoSpin, Thermo, USA), prepared onto infrared (IR) microscope slides and analyzed by IRMSI. The unstained slides were interrogated by a beam of IR light that analyses pixels of 6.25x6.25 μm in size on the sample spot. A 4x4 mm IR image was taken from every sample consisting of 409,600 complete IR spectra, allowing a descriptive and discrete biochemistry of a cell located at each pixel coordinate. Pixels from single cells were added and averaged, resulting in one IR spectrum. Samples were then Pap stained. High-resolution (40x) images were captured from each cell examined (500-1000 /slide). A diagnostic computer algorithm was developed by correlation of spectral and cytological features.

Results: Over 50 IR images were recorded, for a total of 50,000 individual cells. Principal component analysis of the data revealed subtle but reproducible spectral differences for healthy superficial and intermediate squamous cells at different stages of the menstrual cycle, both in pre- and post-menopausal women. These changes were mostly manifested in the protein region of the IR spectrum, indicating a distinct change in the protein composition of these cells. In contrast, more pronounced spectral differences between healthy cells and those showing dysplasia were observed in both the protein and phosphate regions of the IR spectrum. In addition, some morphologically normal-appearing squamous cells within dysplastic sample showed small but identifiable spectral changes that might be precursor to dysplasia.

Conclusions: IRMSI can identify subtle but reproducible biochemical changes within normal and dysplastic squamous cells. This technique may help in the diagnosis of cervical dysplasia when the morphology is equivocal and in the detection of predysplastic changes in normal-appearing cells.

386 Diagnoses of Squamous Cell Carcinoma and Adenocarcinoma of the Lung Using Cytology Specimens: A Retrospective Review with Biopsy Correlation

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Background: In view of the EGFR dependent targeted therapy for adenocarcinoma of the lung, the diagnosis of non-small cell carcinoma (NSCC) on cytology specimens is no longer adequate. There is an increased need to differentiate between squamous cell carcinoma (SCCa) and adenocarcinoma (AdCa) of the lung. The purpose of this study is to determine rate of cytologic diagnosis of adenocarcinoma and squamous cell carcinoma of the lung, correlation of cytologic diagnosis with lung biopsy specimens and as well as to determine the value of cytology in making this distinction.

Design: We retrospectively reviewed all pulmonary cytologic specimens (bronchial washings, bronchial brushings, bronchial alveolar lavage and fine needle aspirations) from 2000 to 2007. Only cases cytologically diagnosed as SCCa, AdCa, NSCC favor SCCa, NSCC favor AdCa and NSCC not otherwise specified were included in the analysis and correlated with corresponding and or follow up biopsy diagnoses.

Results: There are 5296 cytology lung specimens over the 7-year period. 823 (15.5 %) of these were diagnosed as malignant. 659/823 (80%) as NSCC, 103/823 (12.5%) were diagnosed as small cell carcinoma, 22 (2.7%) as metastatic carcinoma, 36 (4.4%) as carcinoma NOS and 3 (0.4%) as carcinoma with neuroendocrine features. Of the 659 cases of NSCC, 214/659 (32.5%) were diagnosed as SCCa/NSCC favor SCCa, 68 (10.3%) were diagnosed as AdCa/NSCC favor AdCa, while the remaining 377 (57.2%) were diagnosed as NSCC NOS. Surgical follow-up for the non small cell carcinoma was present in 196/659 cases (29.7%). All the surgical specimens were biopsies with only 2 lobectomies. There was 82% and 84.6% correlation with the biopsy specimens in SCCa and AdCa respectively.

Table 1. Cytologic diagnoses with follow-up or concurrent biopsies

	SCCa biopsy	AdCa biopsy	NSCC NOS biopsy	Benign bronchial mucosa	Hamartoma
SCCa Cyto (n=67)	55 (82%)	1 (1.5%)	4 (6%)	6 (9%)	1 (1.5%)
AdCa Cyto (n=13)	0	11 (84.6%)	0	2 (15.4%)	0
NSCC NOS cyto (n=225)	39 (17.3%)	47 (20.9%)	110 (48.9%)	29 (12.9%)	0

Conclusions: There is a good surgical pathology correlation between cytologic diagnosis of adenocarcinoma and squamous cell carcinoma. Due to sampling error on biopsy, there is an increased chance for a diagnosis of carcinoma on cytologic specimens. Therefore since cytology may be the initial specimens obtained for diagnosis, subclassification of non small cell carcinoma, may obviate the need to obtain additional surgical biopsy for diagnosis.

387 Cytology of Endobronchial Ultrasound-Guided Transbronchial Needle Aspiration (EBUS-TBNA)

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Background: Endobronchial ultrasound-guided transbronchial needle aspiration (EBUS-TBNA) is a relatively new and minimally invasive technique for diagnosing certain lung diseases and for preoperative staging of lung cancer. During EBUS-TBNA procedure, a dedicated 22-guage needle is used to obtain the specimen. It has been reported that EBUS-TBNA has 69 to 94.6% sensitivity and 100% specificity for staging lung cancers. However, there are no studies in the English literature to investigate the cytologic features of EBUS-TBNA specimens in diagnosing lung lesions. In this study, we have investigated the diagnostic profile of cytology specimens, the correlation between cytology and core biopsy, and the potential cytology diagnostic pitfalls in EBUS-TBNA specimens in diagnosing lung lesions.

Design: A total of 106 cytology cases of EBUS-TBNA were identified in the Johns Hopkins Hospital archives from January to July 2008. Of the 106 cases, 66 cases had a corresponding core biopsy, which was additionally reviewed and correlated.

Results: Patients' age ranged from 17 to 89 years old with a mean age of 58 years old. Male to female ratio was 1 : 0.8. Among the 106 cytology cases, 46 cases (43.4%) were diagnosed as malignant neoplasms, 53 cases (50%) were diagnosed as benign diseases, and 7 cases (6.6%) were non-diagnostic. A total of 66 cases had corresponding core biopsies. Among these 66 cases, 19 (28.8%) were for cancer staging, and 47 (71.2%) were diagnosed with lung lesions. 8 out of 47 cases (17%) showed discordant diagnoses between cytology and core biopsy. In these 8 cases, 3 were diagnosed cancer by cytology and non-diagnostic on core biopsy. These 3 cases were later confirmed to be cancer by surgical resection. 2 cases were diagnosed as granuloma by cytology but had inflammation on biopsy specimen. 2 cases were diagnosed as normal but had granulomas on core biopsy. Only 1 case was diagnosed normal on cytology but was adenocarcinoma on core biopsy.

Conclusions: Our data showed that EBUS-TBNA cytology specimen had 100% adequacy and core biopsy specimen had only 85% adequacy. EBUS-TBNA cytology had 89% sensitivity and 95.7 % specificity in diagnosing lung lesions. Our date also showed that the diagnostic accuracy for granulomatous disease was poor. It may be related to a failure to recognize reactive alveolar macrophages. In addition to staging lung cancer, our findings suggest that EBUS-TBNA cytology is an accurate technique for diagnosing lung lesions.

388 Comparison of Clinicopathologic Features, Preoperative Fine Needle Aspiration, and Expression of Immunohistochemical Markers in Thyroid Papillary Microcarcinoma and Thyroid Papillary Carcinoma

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Background: Thyroid papillary microcarcinoma (TPMC) is defined by WHO as thyroid papillary carcinoma (TPC) smaller than 1.0 cm. Some investigators consider TPMC as "incidental" and clinically insignificant; others believe that TPMC should be regarded as classic papillary carcinoma. In this study, we evaluate the clinicopathologic features, preoperative diagnosis on fine needle aspiration (FNA), and expression of some tumor-associated markers in both TPMC and TPC.

Design: One hundred ninety-three consecutive cases of papillary carcinoma of the thyroid were included and divided into three groups as in Table 1. Immunohistochemical stains for galactin 3, c-Met, S100A4, and S100A6 were performed in 58 cases. The staining intensity and distribution were recorded.

Results: The immunostaining results were diffuse and strong staining for all markers in the majority of cases in G1, G2 and G3. The status of preoperative FNAs and results for G2 and G3 were summarized in Table 2. Seventy-three percents of cases in G2 and 52% cases in G3 received the preoperative diagnosis by FNAs. TPMC accounted for 42% (81/193) of the total cases; and 62% (50/81) of TPMC received the preoperative FNAs.

Table 1. Summary of Clinical Features of 193 Cases

Group	Cases	Age #	F:M	F. Variant*	Size (cm)	Metastasis (%)
G1 (>1.0cm)	113	46	2.3:1	28 (25%)	2.96	36 (32%)
G2 (0.5 - 1.0cm)	37	51	4.3:1	11 (30%)	0.76	6 (16%)
G3 (<0.5cm)	44	52	3.8:1	24 (55%)	0.17	0

#Age = Mean age; *F.variant = Follicular variant

Table 2. Summary of FNA Results in Group 2 and Group 3

Tumor size (cm)	Positive on FNA	Suspicious/atypical on FNA	Negative on FNA	FNA not done
G2 (0.5-1.0, N = 37)	14/37 (38%)	11/37 (30%)	2/37 (5%)	10/37 (27%)
G3 (<0.5, N=44)	7/44 (16%)	9/44 (20%)	7/44 (16%)	21/44 (48%)

Conclusions: These data indicate 1) TPMC expresses similar markers to TPC, including markers associated with unfavorable prognosis; 2) metastasis is present in 16% of TPMC only when a tumor is larger than 0.5 cm; 3) Since TPMC accounts for 42% of the total cases in this study, it should not be considered as "incidental" especially when a tumor is larger than 0.5cm. Therefore, we propose that TPMC should be defined as papillary carcinoma smaller than 0.5 cm, and a tumor larger than 0.5 cm should be clinically regarded as classic TPC.

389 Ki-Ras Mutation Analysis of Pancreaticobiliary Cytology Specimens with Indeterminate Diagnoses

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Background: Fine needle aspiration (FNA) and common bile duct (CBD) fluid cytology have been traditionally used for early detection of pancreaticobiliary cancers. Quite frequently, the cytological interpretations are indeterminate. Mutational activation of Ki-ras oncogene is almost universally present in these cancers. We investigated retrospectively the diagnostic usefulness of detection of Ki-ras mutation in pancreaticobiliary cytology specimens in which an equivocal diagnosis was made.

Design: Cases at a single institution over the past 12 years were retrieved for patients with either pancreaticobiliary FNA or CBD brushing. Excluding patients with definitive malignant diagnoses or insufficient specimens, cases with any degree of suspicion for malignancy, i.e. atypical or suspicious diagnosis, were included. All study cases had a reflex Ki-ras mutation analysis at codon 12/13 using polymerase chain reaction followed by single strand conformational polymorphism analysis. Tissue follow-up was available in all study patients.

Results: A total of 131 patients were identified (Table 1). The follow-up histologic diagnoses included pancreaticobiliary adenocarcinoma (66 cases), IPMN (6 cases), mucinous cystic tumor (1 case), adenoma (5 cases), neuroendocrine tumor (5 cases), focal epithelial atypia (5 cases), chronic pancreatitis (6 cases), and normal histology (37 cases) (Table 1). Overall, the sensitivity and specificity of Ki-ras mutation for the detection of pancreaticobiliary epithelial malignancies (adenocarcinoma, IPMN, and mucinous cystic tumor) were 57% and 93%, respectively. The positive and negative predictive values of Ki-ras mutation for the presence of epithelial malignancy were 91% and 64%, respectively.

Ki-ras Mutation Distribution in 131 Cases

Final Tissue Dx	Ki-ras (+)	Ki-ras (-)	Total
Adenocarcinoma	38	28	66
IPMN/Mucinous Tumor	3	4	7
Benign Adenoma	0	5	5
Neuroendocrine Tumor	0	5	5
Epithelial Atypia	0	5	5
Chronic Pancreatitis	1	5	6
Normal Histology	3	34	37
Total Number	45	86	131

IPMN: Intraductal papillary mucinous tumor

Conclusions: Representing the largest study of its kind, we confirm that Ki-ras mutation carries a high positive predictive value for the presence of epithelial malignancy in patients with an indeterminate pancreaticobiliary cytology. Our results further advocate Ki-ras mutation analysis as an important adjunct to the cytological practice. It should be noted that negative detection does not rule out malignancy in a significant portion of the cases and Ki-ras mutation can be detected in morphologically benign conditions.

390 Integration of Morphology with Fluorescent In Situ Hybridization (FISH) Increases the Accuracy of Bladder Cancer Diagnosis in Standard Papanicolaou (PAP) Urine Cytology: Evaluation of Chromosome Tetrasomy in Urothelial Carcinoma

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Background: UroVysion™ FISH is routinely used to detect bladder cancer. FDA-approved criteria for a positive result are defined as chromosome (CHR) polysomy (POLY) in ≥4 cells. By definition this includes tetrasomy (TET), a natural product of cell division, which is a common factor leading to false positive results. Therefore, we evaluated the utility of separate POLY and TET categories, using published thresholds (TET ≥4, ≥10, and ≥10%), or a combined assay integrating cytomorphology and FISH (CM-FISH), to increase test accuracy.

Design: 133 cases of urine cytology with a PAP-stained slide and associated biopsy were selected. Cytological review resulted in 22 negative, 75 atypical, 25 suspicious and 11 positive diagnoses. 63 cases showed no tumor on biopsy. 43 cases were low-grade and 27 were high-grade by the WHO/ISUP criteria. Urothelial cells with atypical cytological features were subjected to CM-FISH using Duet™/Solo™ Automated Imaging System. TET was defined as 4 copies of CHR 3, 7, 17, 9p21, with up to one missing probe signal (e.g. 4, 3, 4, 4).

Results: The FDA scoring criteria yielded a sensitivity of 84.3% and specificity of 55.6%. Excluding normal cells in the CM-FISH caused a modest reduction in sensitivity to 77.1%, but a marked increase in specificity to 84.1%. Removing TET from the POLY class reduced sensitivity to 67.1% and increased specificity to 71.4%. A separate analysis of cases with <4 POLY cells, showed TET ≥4, ≥10 or ≥10% accounted for 30.4% or 17.4% of positive biopsies. Using CM-FISH with TET ≥10 increased sensitivity to 34.8% and gave a specificity of 97.8% in the same analysis.

Category	POLY+TET		POLY	TET	TET (exclude POLY≥ 4)		
Threshold	≥ 4		≥ 4	≥ 10	≥ 4	≥ 10 (≥ 10%)	≥ 10
Morphology	NA	CM-FISH	NA	NA	NA	NA	CM-FISH
Sensitivity	84.3	77.1	67.1	11.4	30.4	17.4	34.8
Specificity	55.6	84.1	71.4	95.2	84.4	97.8	97.8
Total case #	133	133	133	133	68	68	68

NA= not applicable

Conclusions: Excluding TET from FDA approved criteria increases specificity but at a cost in sensitivity. Based on our data, we recommend using CM-FISH to achieve the highest specificity without losing sensitivity. As criteria for positive FISH, TET alone has a limited value.

391 Mutational Analysis of EGFR in Cytological Specimens of Patients with NSCLC. Usefulness of Papanicolau Stained Smears as a Source of Optimal DNA

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Background: EGFR mutations condition the clinical response of NSCLC to novel chemotherapeutic agents such as Erlotinib and Gefitinib. Cytological specimens are often the only material available, especially in patients with advanced NSCLC. We sought to determine the feasibility of identifying such mutations in tumor DNA extracted from cytological samples in particular from Papanicolau stained slides in patients with lung cancer diagnosed by cytology.

Design: During the last 12 months, oncologists asked for EGFR mutational analysis in 105 NSCLC patients. In 59 of these cases we had only cytological material available. The series consist of 3 pleural and 1 pericardial fluids, 1 BAL, and 54 FNAs (23 lung, 29 mediastinal lymph nodes, 1 bone, and 1 adrenal gland). DNA was obtained from fresh liquid in 5 cases, from cellblock in three, and from Papanicolau stained smears in 51 cases. Cytological diagnosis was adenocarcinoma in 40 cases, 12 were poorly differentiated NSCLC, 3 squamous cell carcinomas, and 4 large cell carcinoma. PCR amplification and direct sequencing of exons 18- 21 of the EGFR gene were performed. Each sample was sequenced in duplicate according to the manufacturer´s specifications in both forward and reverse directions using an ABI prism 310 (Applied Biosystems).

Results: EGFR mutations were identified in 13 patients (22%). In these cases, DNA was obtained from Papanicolau stained smears in 12 cases, and from a pleural fluid sample in one.

Conclusions: Cytological samples can yield adequate material for EGFR mutation analysis, even after being stained, thus providing valuable information which might guide therapeutic decisions in patients with NSCLC. The use of Papanicolau stained smears allows retrospective studies and assures the presence of large amount of tumor cells in the material destined to DNA analysis.

392 Examination of Deeper Levels Is Useful in Identifying High Grade Squamous Intraepithelial Lesions in Cervical Biopsies

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Background: When a cervical Pap smear shows a high-grade squamous intraepithelial lesion (HSIL), colposcopy with cervical biopsy is usually performed to confirm the diagnosis. Should pathologic examination of the initial level of the cervical biopsy fail to reveal HSIL, we routinely review the Pap smear to confirm the diagnosis and we also examine three deeper levels of the cervical biopsy to exclude HSIL deeper within the block. This study was aimed to determine the utility of examining three deeper levels in cervical biopsies to identify HSIL when initial levels are negative in patients with a review-confirmed HSIL on prior cervical Pap smears.

Design: We retrieved from our institutional pathology archives all cases during the period between January 1, 2002 and December 31, 2007 that met the following criteria: 1) a prior Pap smear had review-confirmed HSIL; 2) the initial level of the cervical biopsy showed no HSIL; 3) three deeper levels of the cervical biopsy were examined. The findings on deeper levels of the cervical biopsy were categorized into two groups: (+) HSIL on Deeper Levels and (-) HSIL on Deeper Levels. The histologic findings on deeper levels in each group were correlated with those in the subsequent cone biopsies. Chi-square test was used to compare the proportions of HSIL identified in the cone biopsies associated with either (+) HSIL on Deeper Levels or (-) HSIL on Deeper Levels noted in the prior cervical biopsy.

Results: Of a total of 217 cases that met the abovementioned criteria, 18 cases (8.3%) fell into the group of (+) HSIL on Deeper Levels, and 199 cases (91.7%) comprised the group of (-) HSIL on Deeper Levels. Eight of the 18 patients with (+) HSIL on Deeper Levels and 21 of 199 patients with (-) HSIL on Deeper Levels underwent cone biopsies. HSIL was present in 8 of 8 cone biopsies (100%) following the biopsy finding of (+) HSIL on Deeper Levels and in 7 of 21 cone biopsies (33.3%) following the biopsy finding of (-) HSIL on Deeper Levels ($p<0.001$).

Conclusions: Cases with (+) HSIL on Deeper Levels in cervical biopsies are more frequently associated with high grade squamous intraepithelial lesions in cone biopsies than those with (-) HSIL on Deeper Levels. Routine examination of deeper levels in cervical biopsies appears to be useful in predicting HSIL in patients with a review-confirmed Pap smear diagnosis of HSIL.

393 Are High Risk HPV Types 73 and 82 Being Overlooked in Cervical Cancer Screening?

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Background: Individual viruses of the 100-plus members of the HPV family are defined by DNA sequence homologies that induce type-specific pathologies. Epidemiologic classification, based on 11 case-controlled studies identified 15 HPV types as high-risk types (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 68, 73, and 82) for cervical cancer. Furthermore, the prevalence of high risk HPV types varies with the geographic locations. However, many diagnostic tests do not routinely detect HPV types 73 and 82. Here, we determined the prevalence and distribution of HPV types 73 and 82 utilizing a next generation HPV test that simultaneously detects, types, and quantifies all 15 high risk HPV types.

Design: COMPLeTe Care HPV test simultaneously detects, types, and quantifies all 15 high risk HPV types that are known to cause cervical cancer. The test also detects beta globin as an internal control. This complex multiplex real time PCR test was extensively validated and compared with other methods. The test was then utilized on 2913 patients to determine the prevalence and distribution of HPV types 73 and 82. The majority of this patient population had abnormal Pap smears.

Results: The prevalence of high risk HPV types 73 and 82 is significantly higher in patients with abnormal Pap smears than in patients with normal Pap smears.

Table 1: Prevalence and distribution of HPV types 73 and 82, the two most neglected high risk HPV types in routine cervical cancer screening tests

Cytologic Diagnosis	N	HPV 73 N (%)	HPV 82 N (%)
Normal	817	5 (0.6)	7 (0.8)
ASCUS	1726	60 (3.5)	57 (3.3)
LGSIL	315	23 (7.3)	8 (2.5)
HGSIL	55	4 (7.3)	1 (1.8)
N= number of cases			

Conclusions: Analysis from numerous studies unanimously concluded that 15 HPV types are considered as high risk because of their importance in cervical cancer development. Here, we have shown the prevalence and distribution of HPV types 73 and 82, the two often neglected high risk HPV types in screening test. Our studies justified the importance of adding these two high risk HPV types in routine screening test as they were not only present in patients with ASCUS Pap smears but also in patients with low and high grade Pap smears.

394 A Next Generation HPV Test That Simultaneously Detects, Types, and Quantifies All 15 High Risk HPVs – Type and Distribution of HPV in Cervical Pap Smears

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Background: Reflex high risk (HR) HPV testing is the preferred approach for managing patients with atypical cervical cytology. However, many HPV tests neither detect all 15 HR HPV types nor type them simultaneously. Typing is important to determine both persistent infection and oncogenic potential. While both HPV16 and 56 are HR types, the risk of developing cervical cancer by HPV16 is significantly higher than HPV56 (434x Vs 45x). Thus, complete and type-specific HPV detection increases the sensitivity and specificity of the test and helps clinicians assess the risk for cervical cancer. We have developed and validated a type-specific HPV test and applied on patients with atypical cervical cytology.

Design: COMPLeTe Care HPV test simultaneously detects, types, and quantifies all 15 HR HPV types with an internal control for sample DNA. This complex multiplex real time PCR test has been extensively validated and then utilized as a reflex HPV test on 2206 patients with atypical cervical cytology. This report describes the distribution of type specific HPV in these samples.

Results:

Table 1: Frequency of HR HPV type specific infection in atypical cervical samples

Cytologic Diagnosis	N	HR HPV+ N (%)	Single Infection N (%)	Double Infections N (%)	Triple Infections N (%)	Quadruple Infections N (%)	Quintuple Infections N (%)	Sextuple Infections N (%)	Septuple Infections N (%)	Octuple Infections N (%)
ASCUS	1726	930 (54)	510 (30)	249 (14)	115 (7)	40 (2)	12 (1)	3 (0)	1(0)	
ASC-H	92	74 (80)	48 (52)	17 (18)	5 (5)	2 (2)	2 (2)			
LGSIL	333	259 (78)	121 (36)	72 (22)	38 (11)	22 (7)	5 (1)			1 (0)
HGSIL	55	54 (98)	31 (56)	15 (27)	6 (11)	2 (4)				
N= number of cases										

Table 2: Prevalence and distribution of Type Specific HPV in atypical cervical samples

HPV Types	Odds Ratio (95% CI)*	ASCUS N (%)	LGSIL N (%)	ASC-H N (%)	HGSIL N (%)
HPV16	434	328 (19)	65 (20)	41 (45)	35 (64)
HPV18	248	73 (4)	23 (7)	9 (10)	4 (7)
HPV31	124	138 (8)	25 (8)	10 (11)	5 (9)
HPV33	373	33 (2)	5 (2)	1 (1)	2 (4)
HPV35	74	64 (4)	12 (4)	5 (5)	1 (2)
HPV39	-	156 (9)	30 (9)	10 (11)	8 (15)
HPV45	198	69 (4)	6 (2)	1 (1)	6 (11)
HPV51	66	173 (10)	39 (12)	6 (7)	4 (7)
HPV52	200	136 (8)	20 (6)	11 (12)	4 (7)
HPV56	45	92 (5)	17 (5)	5 (5)	2 (4)
HPV58	115	57 (3)	9 (3)	2 (2)	1 (2)
HPV59	419	125 (7)	18 (5)	7 (8)	8 (15)
HPV68	54	37 (2)	10 (3)	0	1 (2)
HPV73	106	60 (3)	17 (5)	2 (2)	4 (7)
HPV82	-	57 (3)	5 (2)	5 (5)	2 (4)
*Munoz et al, *N Engl J Med* 2003; 348:518-27					
N= number of cases					

Conclusions: HPV type specific testing with COMPLeTe Care revealed a significant number of patients with multiple infections. HPV16 was the most prevalent type. Surprisingly, the prevalence of HPV18 was significantly less than other types. This next generation test has many advantages and sets a new standard in cervical cancer screening that will improve patient management and critically reduce morbidity, mortality, and costs.

395 Evidence-Based Pathology: A Review of "Best Evidence" Supporting the Value of Thyroid FNA To Predict "Risk of Malignancy"

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Background: A National Cancer Institute (NCI) thyroid FNA panel recently recommended standardized diagnostic categories: benign (B), atypia of undetermined significance (US), follicular neoplasm (FN), and suspicious for malignancy (S) with "risks of malignancy" (RM) of <1%, 5-10%, 20-30% and 50-75% respectively.

Design: The "best evidence" supporting these RM was reviewed to determine: evidence level, whether the data were based on clinical follow-up (FU) of most patients in each cohort or on repeat cytology or surgery FU information gathered from selected cases and whether any standard risk estimates such as relative risk (RR) or others were provided.

Results: The "best evidence" cited as references in the NCI guidelines include 4 well-designed level III studies and a review article. These studies reported retrospective experience with 7667 thyroid FNA. None of the studies provided clinical follow-up for a significant number of patients in the B, US and FN categories. Only 1 study provided cytologic follow-up for "B" cases and reports 15 malignancies in 2526 FNA initially reported as B. None of the studies provided RR or other risk estimates.

Conclusions: Current estimates of "malignancy risk" provided by the NCI expert panel probably overestimate the possibility of subsequent thyroid malignancy in patients with FNA showing B, US and FN lesions. Current RM estimates are not based on studies where entire populations were followed-up and may be subject to sample bias as they do not include information from patients lost to follow-up. "Proportions of malignancy" derived from cytology and/or surgical follow-up data should be used as an accurate substitute for "risk of malignancy".

396 Hematolymphoid Malignancies in Serous Effusion Cytology: Cytomorphology, Ancillary Studies, and Diagnostic Pitfalls

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Background: Detection of lymphomas and leukemias in serous effusion cytology is difficult. The current study aims to review the cytomorphology, diagnostic pitfalls, and ancillary studies of serous effusion cytology specimens which were reported as diagnostic of malignant lymphoma or leukemia.

Design: All serous effusion cytology specimens over a 3.5 year period with a diagnosis of a hematolymphoid malignancy were retrospectively reviewed. The cytological material, ancillary studies, and available histological follow-up were analyzed.

Results: Out of the 3892 serous effusion cases, 93 (2.4%) cases were diagnostic of a hematolymphoid malignancy. This included 72 pleural, 19 ascitic, and 2 pericardial fluids. The final diagnoses included: diffuse large B cell lymphoma (n=18); chronic lymphocytic leukemia (n=15); Non-Hodgkin's B-cell lymphoma, not otherwise specified (n=14); plasma cell myeloma (n=13); follicular lymphoma (n=7); acute lymphoblastic leukemia (n=7), acute myelocytic leukemia (n=5); marginal zone lymphoma (n=3), mantle cell lymphoma (n=3), T-cell lymphoma (n=3), Burkitt lymphoma (n=2), Hodgkin's lymphoma (n=1), primary effusion lymphoma (n=1), and post-transplant

lymphoproliferative disorder (n=1). Eighty-four patients (90%) had a previous diagnosis of lymphoma or leukemia, while the remaining 9 (10%) had no history of malignancy. The differential diagnoses in these cases included: reactive lymphocytosis, small cell carcinoma, small round blue cell tumors, poorly differentiated carcinoma, reactive mesothelial cells, and potential mimics of Reed-Sternberg cells. The most helpful cytomorphological features were the presence of non-cohesive, small to intermediate size atypical cells with a scant to moderate amount of basophilic cytoplasm, frequent nuclear irregularities, and apoptosis (best seen in cell block preparations). Ancillary studies were utilized in 61 cases, including flow cytometry in 49 cases, immunocytochemistry in 27 cases, and FISH in 4 cases.

Conclusions: The cytological diagnosis of leukemia and lymphoma in serous effusions is difficult due to the cytologic mimics and pitfalls. Exfoliative cytology is a valuable method for diagnosing hematolymphoid malignancies. Cell block preparations and fresh samples for flow cytometry or FISH studies provide material for ancillary studies which can help to sub-classify these entities.

397 Rapid On-Site Evaluation in the Endobronchial Ultrasound-Guided Fine Needle Aspiration Biopsy of Mediastinal Lymph Nodes

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Background: Endobronchial ultrasound-guided fine needle aspiration biopsy (EBUS-FNA) has been increasingly used in the nodal staging of lung cancer and evaluation of mediastinal lymphadenopathy of other etiologies. Rapid on-site evaluation (ROSE) is a crucial component of the FNA biopsy to assure adequate sampling and appropriate specimen triage. Preliminary diagnosis rendered at the ROSE may also guide clinical management. In this retrospective study, we compared the ROSE diagnosis to the final cytological diagnosis and assessed the impact of ROSE diagnosis on patient management.

Design: A total of 166 mediastinal lymph nodes in 84 patients were biopsied via EBUS-FNA at our institution from August 2007 to July 2008. ROSE was performed by cytopathologists with cytomorphologic analysis on Diff-Quik stained smears. The final cytological diagnosis was rendered by integrating the findings on smears, cell-block, and ancillary studies if applicable. The ROSE and final cytological diagnoses were classified as negative, granuloma, atypical, suspicious or malignant.

Results: Satisfactory EBUS-FNA biopsy was seen in 123 of 166 lymph nodes (74%) and in 56 of 84 (67%) patients. Overall, the ROSE and final diagnoses were precisely correlated in 106 of 123 lymph nodes (86%).

		Final Cytological Diagnosis				
ROSE	LN (n)	Negative	Granuloma	Atypical	Suspicious	Malignant
Negative	55	51 (92%)	2 (4%)	2 (4%)	0	0
Granuloma	18	0	18 (100%)	0	0	0
Atypical	12	3 (25%)	0	4 (33%)	1 (9%)	4 (33%)
Suspicious	6	0	0	2 (33%)	1 (17%)	3 (50%)
Malignant	32	0	0	0	0	32 (100%)

Thirty-seven patients (44%) had surgical follow up with mediastinal lymph node biopsy. Sixteen patients who underwent lymph node biopsy had unsatisfactory EBUS-FNA biopsies. The remaining 21 patients had satisfactory EBUS-FNA biopsies that were diagnosed by ROSE as negative (n=10), granuloma (n=2), atypical (n=5), suspicious (n=2), and malignant (n=2), respectively.

Conclusions: The results suggest that ROSE results correlate with the final cytological diagnoses in most cases, especially in cases with a diagnosis of negative, granuloma or malignant. For the unsatisfactory cases or cases with atypical and suspicious diagnoses, clinical correction is required for appropriate patient management.

398 Evaluation of False Negative Intraoperative Touch Imprint Cytology of Sentinel Lymph Nodes in Breast Cancer Patients

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Background: Intraoperative imprint cytology (IC) of sentinel lymph nodes (SLNs) helps direct surgeons to proceed with axillary lymph node dissection (ALND). False negative (FN) rates of ICs vary between 10-50% in different series. ALND is delayed in FN IC cases, hence the importance of a correct cytologic diagnosis. We analyzed the factors responsible for FN ICs of SLNs and recommend steps to prevent it.

Design: We retrospectively analyzed 342 breast cancer (BC) patients who underwent SLN biopsies (2003-2006). 310 cases had negative IC results, and of these, 33 patients had positive SLN permanent histology. 3 cytopathologists then reviewed these 33 patients having 40 sets of ICs (one Diff-Quik (DQ) and one Hematoxylin-Eosin (H&E) per SLN). The consensus diagnosis at the time of the review was compared with the corresponding positive histology slides. Parameters evaluated included classifying the FN IC results as interpretive errors, BC subtype, diagnosis rendered by cytopathologist (CP) vs. non-cytopathologist (NCP), sampling errors, adequacy of the IC for interpretation, and correlation with the size of the metastatic SLN foci.

Results: IC results in 29/40 SLNs were confirmed negative upon review, while 11 were interpretive errors. 25 SLNs showed micrometastasis, while 15 showed macrometastasis. 25/33 patients had invasive ductal BC, and 8 had lobular BC. 9/40 FN ICs were reported by CPs, while 31 by NCPs. IC in 2 cases was evaluated to be unsatisfactory due to scantly cellular smears. 2 SLNs were partially replaced by tumor metastasis, but the ICs were negative. In 11 SLNs categorized as interpretive errors, the tumor cells were identified in 7 DQ and in all 11 H&E cytologic preps.

Conclusions: Interpretive errors were more common when examination was done by NCPs emphasizing the importance of cytolopathologist's consultation in difficult and negative cases. ICs were more often negative in SLNs with micrometastasis than with macrometastasis, which probably reflects touch preparation sampling. Two SLNs with large foci of tumor metastasis were found negative on IC, underscoring the importance of gross examination of lymph nodes. Poor preparation technique of IC slides provided insufficient material for cytologic examination contributing to FN SLN interpretation. Tumor cells were identified more frequently on H&E stained IC slides emphasizing the importance of nuclear detail in cytologic diagnosis.

399 Fine Needle Aspiration Biopsy Is a Sensitive Diagnostic Tool for Myxoid Lesions: A Cytohistological Review with Emphasis on Ancillary Studies and Diagnostic Pitfalls

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Background: The fine needle aspiration biopsy (FNAB) diagnosis of myxoid lesions in soft tissue and bone can be challenging due to overlapping clinical, radiological, and cytological features. The aim of this study is to review benign and malignant myxoid lesions diagnosed by FNAB, to highlight the diagnostic pitfalls, and to assess the utility of ancillary studies.

Design: Myxoid lesions in the soft tissue and bone, diagnosed by FNAB between January 2004 and December 2007, were retrospectively retrieved and reviewed. The cytological material, ancillary studies (immunohistochemical and cytogenetic/FISH studies), and available histological follow-up were analyzed.

Results: A total of 40 cases (5%) of myxoid lesions were identified from 862 soft tissue and bone FNABs, including 26 (65%) malignant lesions and 14 (35%) benign tumors/lesions. The most common malignant neoplasms were myxoid liposarcoma (n=8), metastatic mucinous adenocarcinoma (n=6), and malignant fibrous histiocytoma/myxofibrosarcoma (n=6). The most common benign entities were myxoma (n=5), myxoid fibrolipoma (n=2) and ganglion cysts (n=2). The diagnostic pitfalls on cytology included the reactive myxoid changes surrounding neoplasms, the edematous background of seroma and the cartilaginous background of chondroid lesions (misinterpreted as myxoid background), in addition to pseudo-lipoblast morphology (misinterpreted as lipoblasts). Immunostains were performed in 19 cases (48%) and cytogenetic/FISH studies were performed in 11 cases (28%). Thirty-six cases (90%) had histological follow-up. The correlation analysis between the cytologic diagnoses and histologic follow-up yielded a sensitivity of 100%, specificity of 82%, and diagnostic accuracy of 94% for the malignant myxoid tumors. The six cases of myxoid liposarcoma accurately diagnosed on cytology were positive for t(12;16) or t(12;22) by FISH or cytogenetic analysis. Two false positive cases were interpreted as myxoid liposarcoma on cytology, but were diagnosed on excisional biopsy as schwannoma and hibernoma.

Conclusions: This study shows that FNAB can be helpful in diagnosing myxoid lesions of soft tissue and bone, particularly when used in conjunction with ancillary studies. The presence of myxoid-like material and pseudo-lipoblasts can make the FNAB diagnosis of myxoid liposarcoma difficult; therefore, this diagnosis should be rendered only in recurrent cases or in new cases with FISH confirmation.

400 So-Called 'Transitional Cell Metaplasia' of the Cervix and Vagina: A Cytologic Study

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Background: So-called 'transitional cell metaplasia' (TCM) of the cervix and vaginal vault has been rarely described, and the cytologic features of only 9 cases of TCM have been reported. We sought to describe the clinical and cytologic features of cases of TCM, with particular reference to potential diagnostic pitfalls.

Design: Cervical and vaginal vault smears reported as TCM or smears from patients with histologically confirmed TCM were retrieved from the files of the Department of Anatomical Pathology, Royal Prince Alfred Hospital, Sydney. The following cytologic features were assessed: cellularity, architecture, cell group thickness, cell shape, nuclear:cytoplasmic ratio, nuclear features, perinuclear haloes and associated pathology. We compared the features of TCM with those of conventional atrophy, high grade squamous intraepithelial lesion (HSIL), reactive endocervical cells and tubal metaplasia.

Results: Six cases (five cervical, one vaginal vault) of TCM were identified from six patients (median age 60 years, range 34 to 80 years). Three patients were postmenopausal, one was perimenopausal, one was pregnant, and one had undergone female-to-male transgender reassignment. In all smears, three-dimensional cell groups in keeping with TCM were identified. They were multilayered and were composed of oval cells with increased nuclear:cytoplasmic ratio and spindle/oval shaped nuclei, with the impression of 'streaming' in some groups. The nuclei showed mild anisonucleosis, mild nuclear membrane irregularities, evenly distributed and variably granular chromatin, and small nucleoli. Nuclear grooves were identified in at least occasional cells in four cases. A surface layer of cuboidal cells was identified in two cases. The background contained associated dysplastic squamous cells (CIN 1 and CIN 2) in three cases, and these were conspicuously different from the cells comprising the TCM. Atrophic changes were present to varying degrees in all cases.

Conclusions: So-called TCM is seen in the setting of atrophy and some authors have suggested that it may represent an unusual morphological manifestation of atrophy. Its cytological differential diagnosis has been reported to include conventional atrophy, HSIL, and tubal metaplasia. In our experience, TCM shows a distinct set of cytologic features which enable its recognition and distinction from these entities.

401 Testing for Mutations in Thyroid Fine Needle Aspiration (FNA) Samples: Clinical Experience with 670 Samples

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Background: Testing for genetic mutations is a novel diagnostic tool that can be applied to thyroid nodules sampled by fine needle aspiration (FNA). The diagnostic value of this test remains not fully characterized. In this study, we report a summary of the results of molecular testing on 670 thyroid FNA samples performed at our institution.

Design: Portions of freshly obtained FNA samples from thyroid nodules were routinely collected for molecular testing. The testing was performed upon completion of cytologic evaluation and was limited to samples diagnosed as suspicious for follicular neoplasms, suspicious for malignancy, follicular lesions of undetermined significance (FLUS), and positive for malignancy by cytology. The panel of mutations included *BRAF* V600E*, NRAS* codon 61*, HRAS* codon 61*, KRAS* codon 12/13 mutations and *RET/PTC1*, *RET/PTC3* and *PAX8/PPARg* rearrangements.
Results: Out of 670 thyroid nodules tested, 72 (11%) were positive for mutations: 37 revealed *BRAF*, 24 *NRAS*, 5 *HRAS*, 2 *KRAS* mutations, 2 *RET/PTC1* and 2 *PAX8/PPARg* rearrangements. Among these 72 samples, FNA cytology was reported as positive for malignancy in 31 cases, as suspicious for follicular neoplasms in 19 cases, as suspicious for malignancy in 11 cases, and as FLUS in 11 cases. Of 60 patients with mutations who have undergone surgery to date, 54 (90%) were diagnosed with malignant tumors. All 32 nodules harboring *BRAF* or *RET/PTC* were papillary carcinomas. Samples positive for *PAX8/PPARg* were oncocytic carcinoma (1) and follicular variant of papillary carcinoma (1). Among 26 *RAS*-positive nodules, 20 (77%) were malignant (18 follicular variant of papillary carcinoma, 2 follicular carcinoma) and 24/26 (92%) were neoplasms (including 4 follicular adenomas), and 2 were hyperplastic nodules.
Conclusions: These results demonstrate that testing for mutations in thyroid FNA samples is feasible and of significant diagnostic value to further refine the cytologic diagnosis. In addition to *BRAF, RET/PTC*, and *PAX8/PPARg* which each had a positive predictive value of 100% for malignancy, *RAS* mutations were common and diagnostically helpful, particularly for the detection of the follicular variant of papillary carcinoma.

402 RAS Mutation in Thyroid FNA Specimens Enhances Predictability of Malignancy in "Follicular Neoplasms"

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Background: In thyroid neoplasms, point mutations in RAS gene (NRAS, HRAS, and KRAS) most commonly occur in follicular derived neoplasms - follicular adenoma (FA), oncocytic adenoma (OA), follicular carcinoma (FC) oncocytic carcinoma (OC), and follicular variant papillary carcinoma (FVPTC) and have been associated with more aggressive behavior. The status of RAS mutation is most applicable to cases in the cytologic category of "suspicious for follicular (or oncocytic) neoplasm" (SFON). The objective of this study is to correlate the status of RAS mutation in "SFON" cases with the surgical pathology outcome.
Design: Thyroid cytology cases diagnosed as "follicular (or oncocytic) lesion" (FOL) were searched from our files for the months from April 2007 to March 2008. During this period, our cytology diagnosis of "FOL" was equivalent to the "SFON" category by the Bethesda 2007 thyroid cytology classification system. For these cases, samples were collected prospectively for cytologic analysis and molecular studies. The material for the molecular studies was collected directly into nucleic acid preservative solution and RAS mutational analysis was performed by the LightCycler real time PCR and post-PCR melting curve analysis. RAS mutational status was correlated with the cytology diagnosis and surgical pathology diagnosis. Fisher exact test was used for statistical analysis.
Results: RAS mutation was found in 11 cases. Surgical pathology correlation was available for all of these cases and revealed the following: FVPTC (7), OA (1), OC (1), FA (1) and nodular thyroid [NT] (1). Ten of 11 (91%) RAS mutation positive cases were neoplastic and of these 8 were malignant (73%). By comparison, 65 cases with surgical pathology correlation were identified to be negative for RAS mutation. These cases revealed the following: papillary carcinoma (12), FVPTC (4), OA (13), OC (4), FA (8), lymphocytic thyroiditis (2) and NT (22). Forty-one of 65 (63%) RAS mutation negative cases were neoplastic and of these 20 were malignant (31%). While the difference in the rate of neoplasia between the RAS positive and RAS negative groups was almost but not statistically significant (p=0.06), the difference in the rate of malignancy between the RAS positive and RAS negative groups was significant (p=0.01).
Conclusions: For cases in the cytologic category "SFON", the presence of RAS mutation indicates a greater probability of malignancy. These results have the potential of refining the clinical management of patients with this cytologic diagnosis.

403 Contribution of Molecular Analysis to Thyroid FNA Specimens with the Diagnosis of "Follicular Lesion of Undetermined Significance"

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Background: By the Bethesda 2007 Thyroid Cytology Classification, "Follicular Lesion of Undetermined Significance" (FLUS) is defined as a heterogeneous category of cases that cannot be classified as benign, follicular neoplasm, or suspicious for malignant cells due to the identification of minimally "atypical" cells, non-specific patterns, or compromising factors including low cellularity, poor fixation, or obscuring elements. The role of ancillary testing has not been determined for this diagnostic category. In this study, we examined and correlated our FLUS cases with the results of our molecular studies and surgical pathology outcome.
Design: Thyroid cytology cases diagnosed as FLUS were searched from our files for the time period from April 2007 to March 2008. For these cases, samples were collected for direct smears, thin-layer processing, and molecular studies at the time of the aspiration procedure. The material for the molecular studies was collected directly into nucleic acid preservative solution and analysis for BRAF and RAS gene mutation and RET/PTC and PAX8/PPARg rearrangement were performed. We correlated the molecular results with the cytology and surgical pathology diagnoses.
Results: Sixty-two cases of FLUS with surgical pathology correlation were identified and subclassified into those with minimal "atypia" (4), low cellularity (15), and non-specific pattern (43). The surgical pathology outcome for these cases revealed the following: benign non-neoplastic – 45 (nodular thyroid – 42; lymphocytic thyroiditis – 2; benign cyst – 1), benign neoplastic – 7 (follicular adenoma – 4; oncocytic adenoma – 3), and malignant neoplastic – 10 (papillary carcinoma follicular variant [FV] – 8; tall cell variant [TCV] – 1; classical papillary carcinoma [CP] – 1). Genetic alterations were identified in 4 malignant neoplasms (BRAF [TCV, CP]-2, RAS [FV]-1, PAX8/PPARg [FV]-1). Overall, 16% of FLUS cases resulted in malignancy and 40% of these cases demonstrated genetic alterations in cytology specimens. All cases with genetic alterations correlated with malignancy.
Conclusions: FLUS specimens most likely represent compromised samples, sampling of non-lesional areas, or neoplasms without the common genetic alterations. Despite the suboptimal and non-specific nature of FLUS specimens, molecular abnormalities were found in 40% of cytology specimens with the outcome of malignancy. In such instances, a repeat fine needle aspiration procedure would not be necessary and resection could be recommended.

404 Increase in the LSIL/HSIL Ratio over Time: A Reflection of Effective Screening

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Background: We have recently proposed a new indicator, the LSIL/HSIL ratio to compare laboratories and populations screened. The aim of this study was to examine this indicator's evolution over time in five institutions with different practice settings, different patient populations and different Pap test (PT) preparations.
Design: We collected the 2001-2007 Pap test data from five institutions using different liquid-based Pap tests (3 Surepath and 2 Thinprep) and calculated the LSIL/HSIL ratio for each year for each institution, as well as the totals for all institutions. We compared the LSIL/HSIL ratio between each successive years and the %LSIL and %HSIL of the total for all institutions for the years 2001-2003 vs. 2004-2007 using the chi-square test.
Results: A total of 1,562,520 were screened at the five institutions during this seven year interval. A total of 62317 cases were diagnosed as LSIL (3.99%) and 9693 (0.62%) as HSIL, with a combined LSIL/HSIL ratio of 6.43. There was a steady increase in LSIL/HSIL ratios for all institutions.

	2001	2002	2003	2004	2005	2006	2007
Inst 1 LSIL/HSIL ratio	4.31	9.68	9.93	15.13	15.86	17.95	21.09
Inst 2 LSIL/HSIL ratio	3.22	3.5	3.34	4.68	6.15	6.29	6.93
Inst 3 LSIL/HSIL ratio	2.1	2.59	3.14	3.2	2.89	3.6	3.45
Inst 4 LSIL/HSIL ratio	3.03	4	4.61	4.11	5.32	6.54	6.22
Inst 5 LSIL/HSIL ratio	4.5	4.67	4.74	6.58	7	7.17	10.15
Total LSIL	6560	7271	7659	7928	8362	12176	12361
LSIL%	3.03%	3.54%	3.93%	4.00%	4.24%	4.56%	4.36%
Total HSIL	1827	1552	1463	1214	1198	1270	1169
HSIL%	0.85%	0.76%	0.75%	0.61%	0.61%	0.48%	0.41%
Total LSIL/HSIL ratio	3.59	4.68	5.24	6.53	6.98	9.59	10.57
Total Paps	216173	205296	195071	198305	197285	267074	283316

The increase was statistically significant ($p<0.05$) for all year-to-year comparisons except for 2004-2005 and was caused both by an increase in LSIL (from 3.49% to 4.34%) and a decrease of HSIL (from 0.79% to 0.51%) from the 2001-2003 to 2004-2007 periods (both $p<0.0001$).
Conclusions: The steady increase in LSIL/HSIL ratios in our institutions most likely reflects a greater efficacy of cytologic screening, resulting in identification of more LSIL and prevention of the progression to HSIL.

405 Immediate Cytological Assessment of Touch Preparations of CT-guided Needle Core Biopsies of Renal Masses Improves Diagnostic Accuracy, Avoiding Repeat Procedures/Unnecessary Surgery

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Background: With increased use of imaging modalities, incidental/ small renal masses are frequently being discovered. Pre-surgical options for diagnosis of renal masses including fine needle aspiration (FNA) and needle core biopsies (NCB) are becoming increasingly important due to both, the awareness that 20-40% of small solid masses are benign and the advent of needle ablative approaches for treatment of renal tumors. Non-diagnostic samples of renal masses using only FNA or NCB, range from 0-24% and 0-21% respectively in the literature. This results in repeat procedures and/ unnecessary surgery. The role of immediate assessment (IA) of touch preparation (TP) of needle core biopsies (NCB) for procuring an adequate sample for diagnosis has hitherto not been studied.
Design: Computer-assisted search for CT-guided NCB with IA (Sept 06- July 08) was performed. On-site touch imprint preparation, staining of the air-dried slides with Diff-Quik stain and IA was performed by the cytopathologist. Adequacy was defined as presence of lesional cells in a renal mass. Parenchyma not likely to represent a mass was called inadequate. Adequacy and diagnosis on IA was compared with final NCB interpretation.
Results: Mean age for 70 patients (41 M, 29 F) in the study was 66.8 years (Range: 29-90y). Size of the lesion ranged from 0.8 cm – 14.0 cm. Number of passes obtained varied from 1-3 (55/ 70) and 4-6 (15/ 70).

Adequacy, on-site versus final diagnosis

	Needle core biopsy diagnosis		
Immediate assessment	Diagnostic	Inadequate for diagnosis	
Adequate	59	4	63
Inadequate	3	4	7
	62	8	70

Table 1

Of the 63 cases called adequate on-site, 59 (93.6%) had diagnostic material and 4 (6.3%) cases were inconclusive for final diagnosis.

Final diagnosis

Diagnostic category	n	%
Malignant	38	54.3
Benign	22	31.4
Suspicious	2	2.8
Non-diagnostic	8	11.4
n	70	

Table 2

Conclusions: 1. TP with IA of NCB was helpful in procuring diagnostic NCB in 93.6% cases. 2. Cytopathologists are in a unique position to make morphological and radiological correlation during on-site assessment to ensure specimen adequacy. 3. Cytomorphological criteria applied to TP were the same as those used for FNA. 4. Normal renal parenchyma can show moderately cellular smears which can initially be misinterpreted as lesional cells/ adequate sample.

406 Evaluation of Renal Neoplasms by FNA and Core Biopsy: Benefits of a Combined Approach To Sampling during Radiofrequency Ablation as Demonstrated by 215 Consecutive Cases

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Background: Radiofrequency ablation (RA) is an acceptable therapy for primary malignancies of the kidney, especially in patients with low stage tumors who may not be good surgical candidates, permitting preservation of renal function. Usually, no pathologic diagnosis of tumor is made prior to therapy. However, at our hospital, pre-RA percutaneous sampling is performed under CT guidance with both fine needle aspiration biopsy (FNAB) and core biopsy (CB).

Design: We report on the complementary roles of these 2 techniques and the advantage of FNAB over CB in a series of 215 consecutive patients undergoing RA for renal neoplasia.

Results: In 88 patients (57%), both specimens were positive for neoplasm. In 13 (9%), both were negative for tumor. In 45 (29%), the FNABs were positive, but the CBs negative. The reverse occurred in 8 patients (5%). When suspicious interpretations by FNAB and CB are added to the calculations, both their complementary nature and the relative higher diagnostic value of FNAB persisted. FNAB positive or suspicious for neoplasm (165 total with concurrent CB), were characterized as renal cell caricnoma (RCC), not otherwise specified (NOS) in 77 cases, RCC clear cell / conventional type in 39, RCC papillary type in 23, neoplasm NOS in 11, RCC with oncocytic features in 5, oncocytoma in 4, angiomyolipoma in 2, and 1 case each of RCC sarcomatoid type, RCC chromophobe type, undifferentiated carcinoma, and metastatic neoplasm. In 14 cases with positive FNABs, the CB allowed a more specific tumor classification. The 8 cases of FNAB considered negative had corresponding CBs that were diagnostic of RCC (6 cases), non-Hodgkin's lymphoma and angiomyolipoma (1 each).

Conclusions: Our findings illustrate the value of the combination of the two biopsy methods for a reliable pretherapy morphologic confirmation of renal neoplasm in that, while FNAB has a relatively greater diagnostic potential and utility for onsite evaluation, the CB provides an additional sample with material for sublassification and additional studies.

407 Atypical Squamous Cells of Undetermined Significance-Cannot Exclude High-Grade Squamous Intraepithelial Lesion: Histology Follow-Up and Clinical Correlation of 455 Cases

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Background: Atypical squamous cells of undetermined significance –cannot exclude high-grade squamous intraepithelial lesion (ASC-H) is a relatively new diagnostic criteria and is suggestive of high-grade squamous intraepithelial lesion (HSIL) and its mimics. In general, ASC-H has a higher positive predictive value than Atypical Squamous Cells of undetermined significance (ASC-US). Pregnant, postpartum or postmenopausal women may have more parabasal cells on their pap smears. Could these parabasal cells cause higher ASC-H interpretation in this population? Do these patients have significant pathology in histological follow-up than the general population? The significance of ASC-H among pregnant, postpartum and postmenopausal populations has been unclear.

Design: A retrospective study was undertaken. The cytopathology files at MetroHealth Medical Center were searched for ASC-H cases from 1/1/2002-6/30/2007. 455 cases were diagnosed as ASC-H during this period. 74 patients were pregnant, 43 patients were postpartum, and 36 patients were postmenopausal. The histological follow-up was recorded and the high grade lesions included CIN II and CIN III. The results were analyzed with SPSS software.

Results: Of a total of 164,457 pap smears reviewed, 455(0.28%) cases were classified as ASC-H. 306 cases had histological follow-up. As a group, 35.9% of patients had HSIL in histological follow-up study, 30% of patients had CIN I, 1.6% had adenocarcinoma, and 32.3% had negative histology. The data was further analyzed by grouping the patients into pregnant, postpartum, postmenopausal and other (non-pregnant, non-postpartum, non-postmenopausal) categories. Postmenopausal women were found to have a significant reduction of high grade lesions in follow-up than the general population, 17.2% and 39.4% respectively (P=0.026). There was no significant difference between pregnant (30%) or postpartum (34.5%) populations compared to the general population (39.4%).

Conclusions: Our study included 455 patents with ASC-H, which compared to the literature, had the most study subjects. An interesting finding in our data was that postmenopausal women had a statistically significant decline in high grade cervical lesions. However, compared to the general population, pregnant and postpartum women do not seem to have a higher incidence of high grade cervical lesions on follow-up. Use of ancillary studies including HPV-DNA may be considered to further define ASC-H in postmenopausal population.

408 Tissue Microarray Immunohistochemical Analysis To Distinguish Contaminating Gastrointestinal Epithelium from Non-Malignant Branch Duct IPMN

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Background: Contaminating GI epithelium presents a diagnostic pitfall in the cytological diagnosis of pancreatic cysts in general, and branch duct (BD) IPMN in particular when sampled by EUS. Accurate diagnosis is vital for proper patient care, which is increasingly non-surgical. Morphology alone is often insufficient to distinguish GI contamination from non-malignant epithelium of these typically low grade mucinous cysts, and misinterpretation can lead to both false positive and negative diagnoses.

Design: Tissue microarrays were constructed using tissue cores from the stomach, duodenum and cyst lining of 15 BD IPMN (6 moderate dysplasia and 9 adenomas) analyzed in quadruplicate with immunohistochemical stains to B72.3, MUC1 core, MUC5ac, MUC2, MUC6, CA19-9, S100P, p16 and CEA. Each core of tissue was individually assessed to account for heterogeneity, and staining was assessed as either positive or negative. ROC curve analysis was performed to determine the optimal immunohistochemical panel to distinguish cyst lining from gastric and duodenal contamination. In ROC analysis perfect tests are associated with an area of 1.0 and completely random tests with an area of 0.5.

Results: The majority of IPMN cores stained positively for S100P (84%), CEA (86%), and p16 (78%), with the first two being excellent markers to distinguish duodenal epithelium from an IPMN. The specificity and sensitivity of S100P and CEA were 91%/86% and 91%/82%, respectively. In distinguishing gastric epithelium from IPMN, ROC analysis showed that all evaluated markers were rated as either fair or poor. Among these, CA19.9 stained 71% of IPMN cores, with a sensitivity and specificity of 72% and 78%, respectively. The table below lists the area under the curve for duodenum versus cyst in the first row and stomach versus cyst in the second row.

Area Under ROC Curve

S100P	CA19-9	CEA	MUC1	MUC2	MUC6	MUC5AC	p16
0.92	0.75	0.86	0.65	0.26	0.72	0.90	0.79
0.58	0.78	0.72	0.33	0.61	0.29	0.48	0.62

Conclusions: Immunohistochemistry staining for S100P and CEA can accurately distinguish non-malignant cyst lining of BD IPMN from duodenal contaminating epithelium, but no marker tested can reliably distinguish gastric epithelium. Given that the majority of BD IPMN occur in the pancreatic head using a transduodenal approach, these data prove useful in the pre-operative differential diagnosis of non-malignant BD IPMN.

409 Anal Cytology: Is There Any Universal Guideline for Adequacy?

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Background: Anal intraepithelial neoplasia (AIN), which may be a precursor of anal carcinoma, has been identified on histology following anal biopsies. The majority of reports describe lesions occurring in homosexual and bisexual men, a group in which there is a known high prevalence of anal condylomata. Anal cytology has recently been proposed as a useful method of identifying AIN lesions. Currently there is no standard guideline for adequacy based on cellularity of the specimen and the presence of transformation zone. Our objective is to compare anal cytology with histology and HPV testing as a method of detecting AIN. We also assess the impact of cellularity of the specimen and presence or absence of glandular cells on diagnosis.

Design: We retrieved anal cytology smears of 80 HIV-seropostive patients from archives of cytology at University Health Network, University of Toronto, between January and August 2008. All samples were collected in cytolyte (Cytyc Corp.). The presence or absence of glandular cells was documented. The cellularity of samples was arbitrarily assessed as ≤ 10, 11-49 and ≥50 squamous cells on 10x objective. These 2 parameters were analysed against cytology interpretation, surgical diagnosis and HPV testing (Digene Corp.).

Results: The patients' age ranged from 29-71 year old (mean of 48). Four of these cases were excluded form study due to lack of concurrent biopsies and HPV testing. 61 of 76 cases had concurrent biopsies and 57 had HPV testing done on the residue of anal cytology samples. Our data revealed that the group of cases with high cellularity (≥ 50) shows higher diagnostic yield in cytology (61.8%), in surgical biopsies (62.3%) and HPV testing (62.7%) in comparison to lower cellularity (≤ 10, 11-49). Also presence of glandular cells improved the diagnostic yield in cytology (71.1%), surgical biopsies(73.8%) and HPV testing (70.1%).

Conclusions: The cellularity and presence of transformation zone elements in anal cytology are essential parameters for more accurate diagnosis. Similar guidelines used for adequacy in cervical cytology may also be applicable in interpretation of anal cytology. Larger series may be needed to establish a universal approach for interpretation.

410 Increased Diagnostic Efficiency of Endoscopic Ultrasound (EUS)-Guided Fine Needle Aspiration (FNA) of Pancreatic Cysts (PC) Submitted in Sure Path™ Liquid Based Medium Using Cell Block Preparation

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Background: Cysts in the pancreas are increasingly being identified due to increased use of high-resolution imaging. They often present a diagnostic challenge given that they may represent malignant or pre-malignant lesions. Cytology of EUS-guided FNA of PC lacks sensitivity but can have a high specificity for mucinous neoplasms and malignancies. However, the method by which cells are prepared and analyzed for cytology may significantly impact how often a diagnosis can be made from a sample. This study investigates the diagnostic efficiency (% of specimens adequate for diagnosis) and the diagnostic accuracy (defined as correlation to final clinical diagnosis) of PC

aspirates evaluated by the SurePath™ liquid based preparation (LBP) (medium used in our laboratory) compared with concurrent cell block (CB) preparation.
Design: Sixty-two cases of FNA from PC with both a LBP and concurrent CB preparation were reviewed by two independent observers. The LBP was evaluated first with subsequent review of the CB with an independent diagnosis. The diagnostic accuracy of each method was then compared with the final clinical diagnosis and cyst-fluid amylase, CEA, and other tumor markers levels.
Results:

Cytologic diagnostic categories (n=62)

Mucinous Neoplasm	14
Intraductal Papillary Mucinous Neoplasm	14
Serous Cystadenoma	2
Pseudocyst	13
Pancreatic Endocrine Neoplasm	1
Benign cyst NOS	1
Abscess	2
Non-diagnostic	15

Table 1

CB were significantly more often diagnostically useful (72.1% diagnostic) than the LBP (34.3% diagnostic) p<0.0001. Of 27 cases where only one preparation was diagnostic, the CB was diagnostic 92.5% of the time whereas the LBP was diagnostic 7.5% of the time. Diagnostic accuracy was extremely high (98%) using both the LBP and the CB. Both the LBP and the CB diagnoses correlated well with cystic CEA levels and the final clinical diagnosis.
Conclusions: Diagnostic efficiency of the CB was far superior to that of the LBP. These results suggest that although the diagnostic accuracy of the LBP and CB are comparable, the diagnostic efficiency of the cell block preparation is significantly better than that of the SurePath™ liquid-based preparation. As submission of a cell block preparation is not currently standard of care, these results have important implications for laboratory practice and suggest that a cell block should always be submitted for evaluation of EUS-guided fine needle aspiration of pancreatic cysts.

411 Anal Intraepithelial Neoplasia (AIN) and HPV in HIV+ Males: A VAMC Report

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Background: HIV patients have higher risk of AIN and anal cancer. Anal cytologic screening on liquid based prep (LBP), automated PCR-based detection of up to 13 types of high risk HPV DNA (hrHPV) to detect HPV, and high resolution anoscopy (HRA) are used. Here we report the efficacy of hrHPV on LBP vs detection of viral cytopathic effect (CPE) in cytology and histology. IHC for P16, p53, p63 and Ki67, adjunctive markers for HPV and diagnosis of HSIL, are studied.
Design: 262 screening anal LBPs from HIV+ men (2005-2007) were assessed by the Bethesda criteria. PCR based hrHPV DNA testing (ARUP labs) was done on 70 LBPs with ASCUS or higher. 34 HRA-guided biopsies were performed; 27 biopsies with sufficient tissue for additional slides were stained with IHC for p16 (BD Pharmagen 1:500), p53, p63 and Ki67 (Dako 1:100, 1:50, 1:200). Two pathologists independently reviewed the cyto- and histopathology. Any discordance was addressed by a joint review of HRA findings and clinical information. ROC curves and areas were generated for each IHC stain.
Results: 262 LBPs analyzed, 87 abnormal: 24 (28%) ASCUS, 42 (48%) LSIL, and 21 (24%) HSIL. HRA guided anal biopsy histology with IHC: 2 (7%) normal, 11 (41%) LSIL, 12 (44%) HSIL, 2 (7%) SCCIS. Tables 1 and 2 summarize the performance of tests.

IHC for HSIL detection with optimal cutoff values

Stain	P16	P53	P63	Ki67
% cutoff	≥15	≥10	≥60	≥10
%Sensitivity	71	43	36	64
%Specificity	85	62	38	61
%PPV	50	43	38	64
%NPV	73	62	36	62
ROCAUC	0.79	0.5	0.45	0.69

70 hrHPV tests: 49(70%) positive, 4 (6%) negative, 17 (24%) "equivocal with scant cellularity.

PCR and LBP performance

	%Sensitivity	%Specificity
LBP	46	78
HPV PCR	82	44

Conclusions: HrHPV testing has high sensitivity, but is limited by low specificity. High prevalence of latent HPV infection HIV+ males limits hrHPV from being a stand alone screening test. Cytology improves the specificity of finding AIN due to direct identification of CPE. HRA guided biopsies are needed for direct visualization and accurate sampling of the lesion. Based on the ROC area under the curves, p16 and Ki67 had the best diagnostic value for identifying HSIL; p53 and p63 were not useful. Using these three tests together will optimize the detection of HSIL and facilitate appropriate management. IHC for P16 and Ki67 could be helpful in discordant cases. Further studies are warranted to confirm our findings.

412 Cytogenetics as an Adjunct to Fine Needle Aspiration in the Diagnosis and Classification of Renal Neoplasms

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Background: Fine needle aspiration (FNA) is an important diagnostic modality for the evaluation and management of selected renal masses. Cytogenetic analysis can serve as a useful adjunct for precise classification because certain kidney tumors are associated with specific chromosomal aberrations; the clear cell, papillary, and chromophobe subtypes of renal cell carcinoma (RCC) are associated with 3p deletions, trisomies of chromosomes 7 and 17, and multiple monosomies, respectively. This study summarizes our experience with the application of cytogenetics as an adjunct to FNA of renal neoplasms.
Design: All renal FNAs from January, 2005 through August, 2008 were identified from the electronic pathology database. The diagnoses were reviewed and correlated with the results of concurrent cytogenetics specimens derived from portions of the FNAs.
Results: 260 FNAs of the kidney were performed during the above time period. Based on an on-site assessment, a portion of the FNA was allocated for cytogenetic analysis in 64 (25%) cases (excluding unsatisfactory and atypical cases). Cultures were successful in 39 of these 64 (61%) cases. The cytogenetic profiles aided in the classification of the tumor in 23 of 39 (59%) cases. Eighteen showed trisomy 7 and 17, twelve via karyotypic analysis of metaphase chromosomes and six via fluorescence in-situ hybridization using probes for chromosomes 7 and 17, and thus were consistent with papillary RCC. Four showed deletions in 3p consistent with RCC, clear cell type. Trisomy 3, seen in a subset of clear cell RCCs, was observed in one case. In the remaining 16 cases, a normal karyotype or non-specific chromosomal aberrations were observed.
Conclusions: FNA of renal specimens is amenable for cytogenetic analysis. Cytogenetics can be useful in the diagnosis and classification of RCCs, especially the papillary and clear cell subtypes.

413 Long-Term Follow-Up of High-Risk HPV(+) Women over 30 with Concurrent Negative Liquid-Based Pap Test (LBPT, Surepath)

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Background: The benefits of the currently recommended strategy of co-screening women > 30 with HPV and Pap tests (PT) are not yet fully known. The aim of this study was to assess the yield of significant cervical lesions (CIN2/3) of women with negative LBPT and a positive high-risk HPV test in a low-risk population screened with Surepath LBPT.
Design: We identified all women ≥30 with negative cytology and concurrent HPV DNA testing performed 2002-2007 on residual Surepath LBPT samples. HPV DNA was detected by PCR using MY09/11 probes and typed by RFLP. HPV+ women, regardless of HPV type, were age-matched with HPV(-) women. The following data were collected: presence of a transformation zone component (TZ), infectious organisms, previous recent abnormal PT, and follow-up PT, biopsies and HPV within up to 72 months (mean 36 months).
Results: 3,958 HPV tests were performed in women over 30. The overall HPV+ rate was 6.8% (268/3958). There was a decline in HPV(+) rates from 8.9% in women aged 30-34 to 3.5% in women ≥70. When only the hr-HPV types included in the Digene HC2 test were considered, there were only 2.5% hr-HPV+ women, and the corresponding decline in positivity for hr-HPV was 4% (30-34 years) to 0% (≥70 years). The 268 HPV+ and 268 age-matched controls had a mean age of 42 ±10.5 (range 30 to 86). The lack of TZ was not significantly different (22% vs. 25%) but HPV+ women had significantly more previous abnormal PT (37% vs. 14.5% OR=3.7 95% CI 2.4 to 5.6, p<0.001) and more infectious organisms (14% vs. 8% OR=1.9 95% CI 1.1 to 3.4, p=0.03). After a mean follow-up of 36 months, 59 women in the HPV+ group and 26 in the control group had cervical biopsies (OR=2.7 95%CI 1.7 to 4.4, p=0.0001). CIN1 was significantly higher in the HPV+ group (OR 9.5 95% CI 2.8 to 31.8 p<0.001) but rate of CIN2/3 were not statistically different between the two groups (p=0.06). HPV+ women with a previous abnormal PT had a significantly higher rate of biopsies of any abnormality and of CIN2/3 (OR=5.9 95% CI 1.2 to 28.99, p=0.03). Only 2 women without a previous abnormal PT results had CIN2-3 (1 CIN2, 1 CIN3).
Conclusions: Our results suggest that co-testing with HPV adds little to LBPT screening of women over 30. Of 3958 women with adequate HPV tests and negative concurrent LBPT, 6.8% were positive for any HPV (2.5% for hr-HPV), but only 9 CIN2/3 lesions were identified in this group after a mean follow-up of 36 months, and 7 of these women had a previous abnormal PT.

414 Detection of Respiratory Pathogens in Bronchoalveolar Lavage Cytology Specimens of Lung Transplant Recipients

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Background: Lung transplant recipients are monitored closely for features of allograft dysfunction. Infection is a frequent reversible cause of allograft dysfunction and its identification an important part of patient management. Bronchoalveolar lavage (BAL) is a commonly employed diagnostic method to detect respiratory pathogens and rule out infectious disease. To assess the diagnostic usefulness of cytologic preparations in the detection of infectious agents, we correlated the results of cytologic examination of BAL specimens by a pathologist with microbiology culture results.
Design: We identified 208 BAL cytology specimens obtained between 1998 and 2007 from 60 lung transplant recipients for which concomitantly microbiology culture results were available. The cytology specimens consisted of a liquid-based ThinPrep preparation, an air-dried centrifuge preparation, and two centrifuge preparations stained with Gomori methenamine silver and a Kinyoun acid fast stain, respectively. The presence of bacteria, fungal hyphae and yeasts, mycobacteria and viral cytopathic effect in the cytology specimens was correlated with the microbiology culture results. Detection of oropharyngeal flora by cytology or culture was not considered pathologic and not counted.
Results: Pathogenic fungi were found in 63 of 208 (30%) cultures. Cytology detected 20 of these 63 (32%) cases. Mycobacteria were found in 9 of 207 (4%) cultures. Cytology detected none of these 9 cases. Pathogenic bacterial strains were found in 16 of 208 (8%) cultures. Cytology detected 4 of these 16 (25%) cases. Viral pathogens were found in 29 of 208 (14%) cultures. Cytologically, a viral cytopathic effect was noted in 2 of these 29 (7%) cases. Only 1 of 208 (0.5%) cytology preparations contained fungal elements while the concurrent microbiology culture did not show any growth. Cytology did not detect viral, bacterial, or mycobacterial agents in any of the cases in which cultures were negative.

Conclusions: Detection of infectious agents in cytologic preparations of BAL specimens has a low sensitivity, and concomitant microbiologic culture should be performed. Cytologic preparations do not increase sensitivity when performed in addition to microbiology cultures. If the difference in turnaround time between cytologic examination and microbiology techniques is less important, ceasing performance of special stains for organisms on BAL cytologic preparations may offer time and cost savings.

415 Follow-Up of ThinPrep Cervical Cytology Cases Diagnosed as High-Grade Squamous Intraepithelial Lesion/Moderate Dysplasia: A Cytohistologic Analysis of 426 Cases Performed over a 5-Year Period

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Background: Cytomorphologic diagnoses are subjective assessments. Under-calling high-grade squamous intraepithelial lesion (HSIL) can lead to delayed diagnoses while over-diagnosis can lead to unnecessary biopsies, increased patient anxiety and increased medical care costs. In this study, the histologic outcome of ThinPrep (TP) cases diagnosed as HSIL/moderate dysplasia (HSIL-mod) was evaluated and the rates and causes of diagnostic discrepancies were determined retrospectively.

Design: A computerized search of our laboratory information system was performed and all ThinPrep (TP) cervicovaginal cytology cases diagnosed as HSIL-mod from July 1, 2003 through June 30, 2008 were identified. All correlating surgical pathology (SP) reports were reviewed. If the follow-up SP demonstrated at least moderate dysplasia, the diagnoses were considered to be concordant. If follow-up SP showed either no evidence of dysplasia (NED) or mild dysplasia, the case was flagged as a discrepancy. All available discrepant cytology and surgical pathology slides were retrospectively reviewed.

Results: During this 5-year period, a total of 127,227 TP smears were evaluated and 426 cases were diagnosed as HSIL-mod (.33%). These patients ranged in age from 15 to 80 (mean: 29). SP follow-up was obtained in 289 of 426 cases (68%) and demonstrated moderate dysplasia, severe dysplasia or malignancy in 160 of 289 cases (55%), NED in 39 cases (14%) and mild dysplasia in 90 cases (31%). Of the 129 discrepant cases with follow-up, the TP and surgicals were available for review in 99 cases (77%). All SP cases diagnosed as NED and 70% of cases diagnosed as mild dysplasia were confirmed; the remaining 30% were downgraded to NED. Of the 99 TP cases originally diagnosed as moderate dysplasia, the review diagnosis was moderate dysplasia in 24 cases (24%), LSIL in 61 cases (62%), ASC-US in 11 cases (11%) and ASC-H in 3 cases (3%).

Conclusions: Almost one-third of patients diagnosed with HSIL-mod (32%) did not undergo follow-up biopsy and only 24% of TP cases diagnosed as HSIL-mod with discordant follow-up surgical biopsies were confirmed as moderate dysplasia. The majority of the discordant HSIL-mod cases (81%) were attributed to cytologic overcalls of LSIL. Careful refinement of our cytologic criteria for the diagnosis of HSIL-mod should reduce the rate of over-diagnosis of this entity and lead to less over-treatment of patients.

416 Expression Analysis of Selected mRNA Is Potentially Useful in the Fine Needle Aspiration Diagnosis of Papillary Thyroid Carcinoma

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Background: Gene expression analysis has identified a series of genes that show consistent differential expression between papillary thyroid carcinoma (PTC) and benign nodules. Genes consistently upregulated in PTC include high mobility group protein gene (HMGA2), keratin 19 (KRT19) and galectin-3 (LGALS3), whereas peroxidase (TPO), deiodinase 1 (DIO1), and trefoil factor 3 (TFF3) are downregulated. In this study, we sought to evaluate whether mRNA analysis of this panel of markers can reliably distinguish benign thyroid lesions from PTC, particularly in the setting of fine needle aspiration (FNA).

Design: Real time quantitative PCR was used to evaluate the mRNA expression of HMGA2, KRT19, LGALS3, TPO, DIO1, and TFF3 in 41 fresh frozen tissue (FFT) and 39 *ex vivo* FNA samples. FFT samples included 4 normal thyroid, 16 follicular adenoma (FA) and 21 PTC, including 2 follicular variant of papillary thyroid carcinoma (FVPTC) and 19 classical PTC (CPTC). FNA samples included 9 hyperplastic lesions (HP), 10 FA, 10 CPTC, and 10 FVPTC. The Ct (PCR cycle number at threshold) value for each gene was obtained and the combined Ct value differences between the 3 upregulated genes (HGMA2+KRT19+LGALS3) and 3 downregulated genes (TPO+DIO1+TFF3) were calculated for each sample, designated as delta Ct. The delta Ct values from the benign and malignant groups were plotted and compared, and a cut-off value was established as a molecular criterion for diagnosing PTC.

Results: In the FFT samples, the molecular criterion accurately classified all PTCs as malignant and 15/16 of the FA as benign, with 1 FA mis-classified as PTC, corresponding to a specificity of 93.7% and a sensitivity of 100%. For the FNA samples, all FA were classified as benign while 15/20 PTC were correctly identified, corresponding to 100%specificity and 80% sensitivity for the diagnosis of PTC. The lower sensitivity in the FNA specimens (vs. in the FFT group) is likely due to contamination of non-neoplastic cells and/or suboptimal RNA yields and quality.

Conclusions: Gene expression of the selected gene panel is useful in distinguishing benign thyroid lesions from PTC. In the setting of FNA, this molecular assay is highly specific for diagnosing PTC but not as sensitive. This limitation in sensitivity is predictable for all morphology-free molecular assays as normal tissue contamination is inevitable in most FNAs. We conclude that this assay can be valuable in helping establish the diagnosis of PTC in cytologically inconclusive FNA specimens.

417 Papillary Pattern in Breast Aspirates; Cytologic Differential Diagnosis with Histopathologic Correlation

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Background: Aim of the study is to analyze the accuracy of nipple discharge/ lavage smears and fine needle aspiration (FNA) for diagnosing papillary lesions, to assess the frequency of their cytologic look-alikes and to correlate the histopathologic features.

Design: Retrospective search was done for cytologic diagnosis of papillary lesions of the breast with subsequent excisional biopsy, over a period of 4.5yrs (2004- 2008) in the database of 3 hospitals in the health system. Of the 278 cases, 165 were FNA performed with ultrasound guidance and 72 FNA without guidance and 41 ductal lavage and nipple secretion smears. Cytology diagnosis were categorized as follows: papillary lesion/ papillary neoplasm (PL/ PN), PL/ PN with atypia, fibrocystic changes with papillary features, papillary carcinoma and correlated with the final pathology on excisional biopsy.

Results: Final pathology showed benign papillomas 157 (56.4 %), papillary duct hyperplasia 4 (1.4 %), FCC 46 (16.5 %), fibroadenoma (FA) 11 (3.9 %), insitu and invasive carcinoma 34 (21.2 %). For detailed results see Table 1.

Table1:

	Papilloma/ Papillomatosis	Papillary Duct Hyperplasia	FCC	FA	DCIS/ LCIS	IDCA/ ILCA
PL/PN	109/ 25	4	36	8	21/ 2	7/ 1
PL/PN with atypia	12/ 2	1	1	0	7/ 1	13/ 1
FCC with papillary features	5/ 3	0	9	3	1/ 0	0/ 0
PCA	1/ 0	0	0	0	2/ 0	3/ 0

X axis - Final diagnosis, Y axis- Cytology diagnosis

PL with atypia; showed 2 cases with papilloma and associated lobular carcinoma in situ (LCIS), 1 showed atypical duct hyperplasia and 1 gynecomastia with focal ductal hyperplasia. 3 LCIS were seen without accompanying papillomas. 11 DCIS showed associated benign papillomas, 5 showed micropapillary type DCIS, 8 intraduct cystic papillary carcinoma and 2 intraduct solid papillary carcinomas. Invasive duct carcinoma (IDCA) showed 2 colloid carcinomas of which 1 had associated benign papillomatosis. 1 invasive lobular carcinoma (ILCA) also showed associated papillomatosis.

Conclusions: Papillary lesions of breast can be reliably diagnosed by cytologic evaluation. Significant overlap of cytologic features exists between benign non papillary and benign and malignant papillary lesions. Since 21.2% of lesions showing papillary features on cytology proved to be malignant, all cases reported as papillary on cytology should have histologic assessment.

418 Cytopathology Glossary: Evaluating Accessibility to Definitions of Descriptive Cytopathology Terms in Textbooks and Internet References Commonly Used by Pathology Residents

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Background: Descriptive cytopathology terms are commonly encountered by pathology residents at departmental and national conferences, in the literature, and while signing out cases with attending pathologists. The meanings of these terms are often not self-evident. For proper understanding, a beginning pathology resident must then seek out the definitions in available reference textbooks or internet search engines/websites. Often a considerable amount of time is spent searching through multiple books and/ or websites to find a certain term, if it can be found at all. In this study, we evaluated the accessibility to the definitions of many commonly used descriptive cytopathology terms in current cytopathology textbooks and internet sources to better determine if the educational needs of trainees are being met.

Design: 12 cytopathology references in total were evaluated including 9 commonly used cytopathology textbooks, and 3 well known internet search engines/websites. 89 descriptive cytopathology terms were searched for in the index and then cross-referenced to determine if the respective definition was present in the text. For internet sites, terms were entered into the search line and the definitions were obtained from following links to the first 20 hits. Percentages of the overall availability of terms present were calculated, as well as the percentage for each single reference.

Results: Of 89 pre-determined cytopathology term definitions, only 32.3% were available over the 12 references used in this study. When using strictly textbooks, 30.8% of the definitions were found. With internet searches, definitions were available 36.8% of the time. One internet search engine provided the highest overall availability with 67.4%. The highest accessibility percentage amongst a single textbook was 56.2%.

Conclusions: Our results indicate that residents must spend a significant amount of time searching the literature to access definitions of commonly used descriptive cytopathology terms. No single source contains a large majority of terms on our list. Although these terms are mentioned frequently in the literature and in practice, the true meaning may not be clear to the inexperienced trainee after consulting multiple sources. We believe there is an educational need for sources to more completely define the meaning of these commonly used descriptive cytopathology terms, many of which serve as a "word picture" to characterize cytologic findings.

419 Utility of EMA, XIAP, and GLUT-1 for the Diagnosis of Malignant Mesothelioma in Body Cavity Fluids

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Background: The distinction between reactive mesothelial cells and malignant mesothelioma (MM) can be challenging in body cavity fluids. A number of immunohistochemical markers have been proposed to assist with this distinction. Epithelial membrane antigen (EMA) is a promising marker of malignancy but published data reveal conflicting results. Recently, X-linked inhibitor of apoptosis protein (XIAP) and an isoform of glucose transporter (GLUT-1) have been proposed as helpful markers

on tissue specimens. This study investigates the effectiveness of EMA and the newer markers XIAP and GLUT-1 to distinguish between reactive and malignant mesothelial cells in cytologic samples.

Design: 73 cases were examined, including 35 cases of MM and 38 cases of benign effusions. The diagnosis was confirmed by histology in all cases of MM. The benign effusions were caused by a variety of non-malignant etiologies in patients with no history of malignancy. Immunohistochemical studies were performed on cell block material from body cavity fluids following heat-induced epitope retrieval (except EMA) using antibodies to EMA (clone E29), XIAP (clone 48/hILP/XIAP), GLUT-1 (clone SPM498, GLUT-1m; rabbit polyclonal, GLUT-1p) and an Envision+ (Dako) detection system. The results were graded using five categories based on the percentage of cells staining: negative (0%), 1+ (<25%), 2+ (25-49%), 3+ (50-74%), and 4+ (75-100%) staining.

Results: At the level of 2+ or higher (≥25% cells stained), EMA, XIAP, GLUT-1m and GLUT-1p have a sensitivity of 74%, 66%, 40%, 67%, and a specificity of 97%, 58%, 95%, 74%, respectively. At a higher threshold (3+ or higher, i.e., ≥50% cells stained), they demonstrate a sensitivity of 71%, 46%, 34%, and 51%, and a specificity of 100%, 79%, 95%, and 92%, respectively. Compared to XIAP and GLUT-1, EMA demonstrated better sensitivity and specificity in distinguishing malignant from benign mesothelium, with an area under receiver operating curve (ROC) (AUC) of 0.91, significantly higher than seen for XIAP and GLUT-1m ($p<0.05$) (Table).

Conclusions: Compared to XIAP and GLUT-1, EMA is a better marker of malignancy for mesothelioma in body cavity fluids, with high specificity and moderate sensitivity.

Table: Comparison of ROCs among different markers

	EMA	XIAP	GLUT-1m	GLUT-1p
ROC (AUC)	0.91	0.67	0.74	0.80
+/- SD	0.039	0.063	0.059	0.052

420 The von Hipple-Lindau Gene Product (Pvhl) Is a Useful Marker in Differentiating Salivary Acinar Cell Carcinoma from Oncocytoma

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Background: The von Hipple-Lindau gene product (pVHL) has been linked to the carcinogenesis of both hereditary and sporadic clear cell renal cell carcinomas. Our recent study demonstrated that loss of expression of pVHL was observed in pancreatic intraepithelial neoplasia (PanIN) and invasive ductal adenocarcinoma of the pancreas (Lin et al. AJSP 2008;32:78-91). In addition, loss of or reduced expression of pVHL has been demonstrated in malignant salivary gland epithelial neoplasms in a small number of cases. In this study, we further investigate the utility of pVHL in the distinction of acinar cell carcinoma from oncocytoma.

Design: We immunohistochemically evaluated the expression of pVHL in 14 cases of acinar cell carcinoma (7 cases from surgical specimens, 5 cases from small biopsy specimens, and 2 cases from cell blocks of fine needle aspirates) and 5 cases of oncocytoma on surgical specimens. The majority of cases also contained normal salivary gland tissue. The staining intensity was graded as weak or strong. The distribution was recorded as negative (less than 5% tumor cells stained), 1+ (5-25% of tumor cells stained), 2+ (26-50% of tumor cells stained), 3+ (51-75% of tumor cells stained), or 4+ (more than 75% of tumor cells stained).

Results: The results demonstrated a membranous and cytoplasmic staining pattern of pVHL in 5 of 5 cases (100%) of oncocytoma, with diffuse staining (3+ or 4+) in all cases. In contrast, only 1 of 14 cases of acinar cell carcinoma was positive for pVHL. Normal ductal cells were positive for pVHL, and acinar cells were negative for pVHL in all cases. The pVHL-positive acinar cell carcinoma case showed oncocytic changes and had no evidence of metastasis after 2-year followup. The original diagnosis on this case is questionable.

Conclusions: The results demonstrate that pVHL is a useful marker in the distinction of acinar cell carcinoma from oncocytoma on both small biopsy and FNA specimens. A large series of cases is needed to confirm the above findings.

421 HPV Testing of LSIL-H: Is There Any Correlation with Positive Predictive Value for HSIL?

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Background: The diagnosis of "low-grade squamous intraepithelial lesion (LSIL), cannot rule out high-grade squamous intraepithelial lesion (HSIL)" or LSIL-H is rendered in cases with unequivocal LSIL and rare "atypical squamous cells, cannot exclude high-grade lesion" (ASC-H). The LSIL-H diagnostic category is not recognized by the 2006 Bethesda Guidelines but is used in cytopathology practice. The positive predictive value (PPV) of LSIL-H for HSIL has been shown by previous reports to be intermediate between LSIL and HSIL and similar to ASC-H. However, only a rare study has evaluated the impact of human papilloma virus (HPV) DNA testing on the PPV of LSIL-H for HSIL. The goal of the current study was to determine whether HPV testing of LSIL-H diagnoses can improve the PPV for high grade lesions.

Design: All cases with the diagnosis of LSIL-MORE/LSIL-H (n=300), histological and/or cytological follow-up within one year (n=220), and hybrid capture 2 HPV testing (n=90) spanning a 36-month period were retrieved. P-values were determined using the ANOVA test statistic.

Results: The PPV of LSIL-H for a high grade lesion on either histological or cytological follow-up was 16.4%.

Follow-up diagnoses after initial LSIL-H

Diagnosis n=220	Percentage (n)
HSIL	16.4% (36)
LSIL	39.5% (87)
ASCUS	6.8% (15)
NEGATIVE	37.3% (82)

Eighty percent of LSIL-H diagnoses were HPV(+). When stratified by diagnostic category, a higher proportion of patients with subsequent HSIL diagnosis were HPV(+) (92.3%) compared to those with either LSIL (85.7%), ASCUS (81.8%) or negative (71%) follow up; the difference, however, was not statistically significant.

HPV positivity by follow-up diagnosis

Follow-up Diagnosis n=90	% (n)	% HPV+ (n)	P-value
HSIL	14.5% (13)	92.3% (12)	p=0.301
LSIL	31.1% (28)	85.7% (24)	
ASCUS	12.2% (11)	81.8% (9)	
NEGATIVE	42.2% (38)	71.0% (27)	

Conclusions: Although a large majority of our LSIL-H cases are positive for high-risk (HR)-HPV, they do not correspond to a higher grade lesion on follow-up. Testing for HR-HPV therefore does not appear to improve the PPV of LSIL-H for high grade follow-up lesions. Furthermore, we conclude that patients with LSIL-H should be managed like patients with LSIL as the majority of cases result in low-grade or negative follow-up.

422 Classification of NSCLCs by Fine Needle Aspiration Biopsy Combined with Core Needle Biopsy: How Specific Can We Be?

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Background: The recent introduction of new, targeted agents for lung cancer treatment has changed oncologists' expectations about pathologic diagnosis of non-small cell lung carcinomas (NSCLCs). Since decisions about treatment options are based on the histologic type of NSCLC, specific morphologic categorization of NSCLCs is necessary, rather than the more general interpretation of "non-small cell carcinoma." Our goal was to assess the frequency with which specific morphologic classification could be accomplished by fine needle aspiration biopsy (FNAB) and concurrent core needle biopsy (CNB).

Design: 34 NSCLCs were evaluated by three cytopathologists. For all cases, FNAB and CNB samples were reviewed and classified using the World Health Organization scheme. A consensus diagnosis was determined, with all pathologists blinded to the diagnosis of the subsequent surgical specimen. The consensus diagnoses were compared with the subsequent surgical excisional material, which was considered the gold standard diagnosis for each case. The Fisher exact test was used to determine significance.

Results: In 29 cases, diagnostic material was obtained by FNAB; five other cases were classified as "atypical" or "non-diagnostic." 27/29 (93%) cases were classifiable as a specific histologic type of NSCLC, and matched the classification of the corresponding resection specimen. The addition of a CNB allowed only 1 more case (97%) to be correctly and specifically classified, a rate that was not significantly different than FNAB alone (p=0.99). The two cases in which the consensus FNAB and subsequent surgical pathology diagnoses differed were an adenocarcinoma with hepatoid features and a pleomorphic carcinoma.

Conclusions: FNAB can achieve specific morphologic categorization for most NSCLCs. CNB offers a small, but non-significant, increase in the number of cases for which specific classification is possible. Uncommon histologic types of NSCLCs may be less likely to be accurately classified by FNAB.

423 Identification of Molecular Alterations Leading to Malignancy in Ductoscopically Procured Mammary Epithelial Cells

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Background: Mammary ductoscopy is a new endoscopic technique that has the potential to allow the direct sampling and analysis of a pure population of mammary epithelial cells. We are taking advantage this new technique to obtain epithelial cells along tumor-containing mammary ducts. This will allow us to define a geographic map of the genetic alterations leading to malignancy in breast cancer.

Design: Our study population consists of women undergoing mastectomy for invasive breast cancer. Cytology samples are procured intraoperatively by mammary ductoscopy from ducts leading to the tumor, and ducts opposite the tumor. Immediately after removal of the breast, a set of snap-frozen tissue samples are harvested from the same regions as well as from the tumor mass. Cytology samples are depleted of contaminating macrophages using magnetic bead technology and the presence of malignant cells is confirmed by a cytopathologist. Tissue samples are examined histologically and microdissection performed on corresponding cryosections. Nucleic acids are extracted from all cytological and microdissected tissue samples. DNA and RNA are subjected to array CGH and gene expression array profiling, respectively.

Results: Twenty-one patients undergoing mastectomy have been recruited to date. Our results indicate that 80% (17/21) of patients had a successful ductoscopy/ductal lavage procedure. Malignant epithelial cells are present in 35% (6/17) of lavage samples on cytological analysis. Preliminary array CGH data shows an increasing number of genetic alterations in epithelial ductal cells going from the nipple towards the tumor. Furthermore, genetic abnormalities can be identified in ductal cells sampled close to the nipple in some patients.

Conclusions: Mammary ductoscopy is a useful technique for procuring epithelial cells from breast cancer patients. In at least one third of our cases, malignant epithelial cells were retrieved. Comparison of the genetic profile of these cytology samples with microdissected tissue samples obtained from the same regions of the duct system is enabling us to evaluate this new technique. This study will result in a better understanding of the geographic location of genetically abnormal cells within the breast.

424 p16^{INK4a} Immunocytochemistry as an Adjunct to Cervical Cytology – Potential Reflex Testing on Specially Prepared Cell Blocks from Residual Liquid Based Cytology (LBC) Specimens

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Background: p16^{INK4a} (p16) is a recognized marker of HPV related dysplasia in cervical biopsies. However, its application to cervical cytology specimens has not been evaluated methodically. The main challenge in applying p16 to cytology specimens is commonly associated, non-diagnostic, cytoplasmic immunoreactivity, which envelops and obscures the nuclear immunoreactivity in uncut cells. We standardized a protocol to overcome this and other obstacles. We evaluated the role of p16 immunoreactivity in residual LBC [SurePath] specimens after cytology interpretations.

Design: Out of 120,000 cases, residual cytology specimens were available for prospective evaluation in 133 [50 LSIL, 28 HSIL, 21 ASC-H, 14 ASCUS and 20 negative (WNL)] cases for preparing HistoGel™ cell blocks. We standardized a method which included a centrifugation step to align randomly dispersed loose cells in LBC specimens along the flat cutting surface. In addition, a visible marker was embedded to monitor the depth while section cutting. This marker also helped serve as a landmark during interpretation of p16 (clone E6H4, mtm laboratories AG) immunostained slides. Only nuclear immunoreactivity in squamous cells was considered positive. Biopsy was considered positive if CIN1 or above (confirmed with p16 in equivocal cases).

Results: Adequately cellular cell block sections showed positivity in 20/28 HSIL, 11/ 21 ASC-H, 13/ 50 LSIL, 0/14 ASCUS and 0/20 WNL. All 7 biopsy positive ASC-H cases were positive for p16 on cytology specimens.

Correlation of follow-up results with p16 immunocytochemistry

Group		T	W/ Bx F/U - P/N*	Sensitivity*	Specificity*	W/ PAP F/U - P/N*	No/ F/U
LSIL	p16P	26% T=50	44%/56% T=27	85%	100%	7%/93% T=14	9
HSIL	p16P	71% T= 28	69%/31% T=16	100%	67%	43%/57% T=7	5

W/, with; Bx, biopsy; F/U, follow-up; p16P, positive for p16; T, total, P, positive, N, negative. Other abbreviations are as per Bethesda Terminology. *Calculated by including only cases with unequivocal biopsy results and cases with adequately cellular specimens for cell blocks. Trouble shooting of false P + N cases showed sampling and biopsy interpretation issues.

Conclusions: 1. p16 on cervical cytology specimens showed excellent correlation with biopsy results. 2. In cases with abnormal but without unequivocal HSIL cytology (such as ASC-H, LSIL, & ASCUS), *reflex p16 immunostaining* using a properly standardized protocol to prepare cell block sections of cervical cytology specimens is recommended.

425 Evidence-Based Pathology: "Risk of Malignancy" Estimates Based Solely on Cytology and/or Surgical Follow-Up May Compromise the Forecasting Accuracy of Thyroid FNA

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Background: A National Cancer Institute (NCI) thyroid FNA panel recently recommended the diagnostic categories: unsatisfactory (U), benign (B), atypia of undetermined significance (US), follicular neoplasm (FN), and suspicious for malignancy (S), estimating "risks of malignancy" (RM) of <1%, 5-10%, 20-30% and 50-75% respectively.

Design: Our one-year experience with 927 thyroid FNA was reviewed using NCI diagnostic criteria. The number of malignancies diagnosed on follow-up FNA and/ or surgery was used to calculate malignancy proportions in percentages. Malignancy proportions and relative risk (RR) of malignancy were calculated by NCI category using two denominators: entire cohort and cases with cytology or surgical follow-up information. Results obtained by the use of the two denominators and paired by NCI category were compared with chi-square statistics.

Results: Malignancy proportions and RR by NCI category using the 2 denominators are listed in Table 1. The differences in malignancy proportions and RR calculated using both denominators are significant for all categories (p=0.01).

Malignancy Estimates for NCI categories for the Entire Cohort and the Patients with follow up

Category	Malignancy Estimates	Entire Cohort	Cases with cytology/Surgical follow up
B	% Malignancy	0.9	6.0
B	RR	0.5	3.8
US	% Malignancy	3.0	21.00
US	RR	0.5	1.4
FN	% Malignancy	12.5	40.00
FN	RR	0.6	1.25
S	% Malignancy	20.00	100.00
S	RR	Not calculated*	100.00
US	% Malignancy	1.4	100%
US	RR	0.5	Not calculated*

*All the patients followed up developed malignancy

Conclusions: Our findings suggest that the "risk of malignancy" estimates provided in the NCI guidelines for thyroid FNA are amplified by the use of cytology or surgical follow-up information as the denominator in calculations. This method does not account for cases that have been lost to follow-up. Most studies in the literature have used this methodology, probably overestimating "risk of malignancy". This problem needs to be considered in future studies.

426 Clinical Trials of the FocalPoint GS System Show Significant Improvements in Sensitivity for the Detection of Squamous Intraepithelial Lesions When Compared to Manual Screening

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Background: Location-guided screening of cervical cytology specimens is a significant advance over routine manual screening. In order to determine if use of the FocalPoint GS System (BD/TriPath) is more effective that manual screening, a large clinical trial was performed.

Design: A 2-armed, masked, adjudicated trial, using SurePath slides (BD/TriPath) was run at 4 clinical sites. The control arm (CA) consisted of routine manual screening and quality control (QC) rescreening, and the experimental arm (EA) consisted of screening by the FocalPoint GS, cytotechnologist review of up to 11 fields of view (FOV), with signout as negative or escalation to full manual screening, QC rescreening, and pathologist review as appropriate. All positive, discordant, and a subsampling of negative slides were adjudicated to a reference diagnosis. The results obtained in the two arms were compared to the reference diagnoses and sensitivity, specificity, and negative predictive value (NPV) were calculated for ASC-US+, LSIL+ and HSIL+ groups.

Results: 12,313 slides were evaluated. The detection sensitivities for HSIL+ were 85.3% (EA) and 65.7% (CA) ($p<0.0001$); and for LSIL+ were 86.1% (EA) and 76.4% (CA) ($p<0.0001$). For ASC-US+, the sensitivities were not statistically different between the study arms at 58.1% (EA) and 59.2% (CA). Specificities were slightly greater in the CA for HSIL+ (97.7% (CA), 95.0% (EA) ($p<0.0001$)) and for LSIL+ (90.5% (CA), 85.6% (EA) (NS)), but slightly greater in the EA for ASC-US+ (82.5% (CA), 84.3% (EA) (NS)). NPV (CA/EA) for not-HSIL+ were 97.7/98.9% and for not-LSIL+ were 98.4/99.0%.

Conclusions: Use of the FocalPoint GS System significantly improved the sensitivity for detection of the important categories of squamous intraepithelial lesion (SIL) and cancer with a much smaller decrement in specificity. Detection of ASC-US+ cases was statistically equivalent, and improved SIL+ NPVs show performance of the device improves accuracy for clinically important entities without increasing equivocal case detection. Use of this device has the potential to significantly improve both accuracy and efficiency.

427 Novel HPV Tests: A Comparative Analysis of the Invader HPV and COMPLeTe Care HPV Genotyping Assays in Low Grade Cervical Cytologic Abnormalities

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Background: High risk (HR) HPV testing is being used increasingly in screening and triaging of women with low grade cervical abnormalities in pap smears. Early indications exist that type specific HPV testing may provide useful information for better stratification of these women. The Invader HPV assay (INV) (Hologic Inc, Bedford, MA) is a recently developed pooled analyte specific reagent for detecting 14 HRHPV with additional probes for typing HPV16 and 18; while the COMPLeTe Care HPV assay (Physicians Reference Laboratory, Overland Park, KS) (CC) is a real time multiplex PCR test that simultaneously detects and types all 15 HRHPV. This study describes performance characteristics of the two tests.

Design: 73 residual SurePath samples (46 ASC-US and 27 LSIL) were tested for HR HPV by the INV and CC assays. Appropriate positive and negative controls were run. Additionally, both assays contained an internal beta-globin control for sample DNA. The analytic sensitivity of the INV HPV 16 assay is ≤625 copies/reaction and HPV 18 of ≤5000 copies/reaction. The analytic sensitivity of CC is <10 copies of HPV/ reaction, and corresponds to <125 copies/ml of Pap samples. Results obtained by the two assays were correlated.

Results: Results are summarized in table 1.

Table 1: Comparison of the Invader (INV) and COMPLeTe Care (CC) Assays for Detection of High Risk HPV

	INV	CC
Assay Method	Invader Technology	Multiplex PCR
Type	Two step test (initial screening wtih selection for HPV16/18 typing)	One step test
HRHPV Positive	49 (67%)	53 (73%)
Multiple Infections	NA	28 (38%)
HPV 16	9 (12%)	10 (14%)
HPV 18	4 (5%)	6 (8%)
Common HPV Types Detected	NA	HPV 51, 56, 59 (19% each); HPV 39 (18%)

NA, not applicable

Conclusions: CC and INV assays showed good (85%) concordance. Differences may be explained by the greater analytic sensitivity of CC. CC offers additional advantage of a one-step type specific information with capability of future determinations of virus persistence, significance of presence of multiple infections and significance of HPV types other than 16 and 18; information that may be essential for personalized management of patients.

428 Is Follicular Adenoma with Equivocal Nuclear Atypia the Precursor Lesion of Encapsulated Follicular Variant of Papillary Thyroid Carcinoma? Fine Needle Aspiration, Histology and Molecular Study

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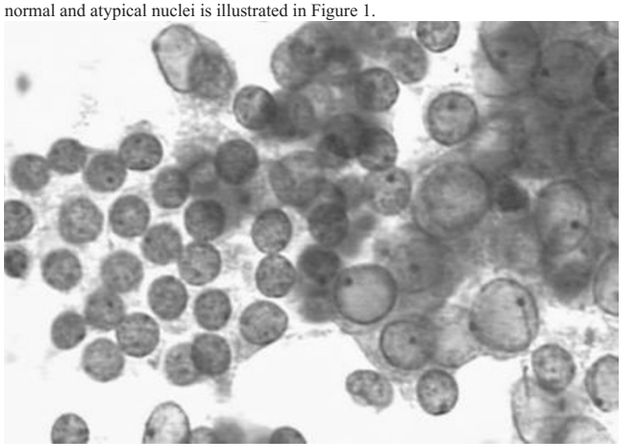
Background: It has been shown that 2% of thyroid malignancies arise within a preexisting benign thyroid nodule and 10% of surgically excised follicular tumors have equivocal nuclear atypia, which may be biologically borderline between follicular adenoma (FA) and encapsulated (E) follicular variant (FV) of papillary carcinoma

(PC). In addition, molecular tumor markers have been identified in some histologically classified FA. In this study we compare those tumors with EFVPC and analyzed their cytological and molecular characteristics.

Design: Fine needle aspiration (FNA) of 27 cases of EFVPC (category 1) and 14 cases of FA with equivocal nuclear features of PC (category 2) were reviewed for the nuclear features. 3 cases from each category were analyzed for BRAF V600E, NRAS 12/13, NRAS 61, KRAS 61, HRAS 12/13, HRAS 61 mutations by DNA sequencing following microdissection.

Results: In category 1, 22 patients were female and 5 were male, age ranged from 24 to 70 years (mean 42), nodule size ranged from 0.7 cm to 4.6 cm (mean 2.3). The PC nuclei were focal in 17 (63%) cases and diffuse in 10 (37%) cases. A case with mixed normal and atypical nuclei is illustrated in Figure 1.



The result of molecular study is shown in Table 1.

Table 1: Encapsulated follicular variant of papillary thyroid carcinoma

	BRAF V600E	N-RAS 12/13	N-RAS 61	K-RAS 61	H-RAS 12/13	H-RAS 61
Case 1	(-)	(-)	(-)	(-)	(-)	(-)
Case 2	(-)	(-)	(-)	(-)	(-)	(-)
Case 3	(-)	1265G>C	(-)	(-)	(-)	(-)

In category 2, 13 patients were female and 1 male, age ranged from 21 to 82 years (mean 45), nodule size ranged from 0.8 cm to 4 cm. The PC nuclei were focal in 10 (71%) cases and diffuse in 4 (29%) cases. The result of molecular study is shown in Table 2.

Table 2. Follicular adenoma with equivocal nuclei

	BRAF V600E	N-RAS 12/13	N-RAS 61	K-RAS 61	H-RAS 12/13	H-RAS 61
Case 1	(-)	(-)	(-)	(-)	Poor amp.	Poor amp.
Case 2	(-)	(-)	(-)	(-)	(-)	(-)
Case 3	(-)	(-)	(-)	(-)	1266G>GC	(-)

Conclusions: More PC nuclei were present in FNA of EFVPC than FA with equivocal nuclei, but it is a continuum. EFVPC were similar to FA with equivocal nuclei in that they showed occasional RAS mutation and no BRAF mutation. Our findings supports the proposal of FA with equivocal nuclei possibly could be the precursor to EFVPTC, a biological continuum from FA to EFVPTC may exist. Molecular analysis of additional cases for each category are necessary to verify this pilot study.

429 UroVysion™ (UroV) "False Positives": True False Positives or Anticipatory True Positives? The University of Pittsburgh Experience

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Background: UroV was developed to increase sensitivity for detecting urothelial neoplasia (UN) over urinary cytology (UC) alone. We have occasionally encountered pts with UroV+/UC-, who later developed a positive UC / bx. The aim of this study is to investigate characteristics of UroV+/ UC- cases.

Design: We retrospectively reviewed 667 UC cases assessed by UroV (Vysis; Downer's Grove, IL) from 9/20/04 to 3/31/07. Subjects include: 1) pts under surveillance for UN with any UC dx and 2) pts screened for UN with an atypical / suspicious / positive UC. UC cases were classified as (+) (suspicious / positive) and (-) (negative / atypical). UroV was prepared from residual urine and scored manually by a Nikon fluorescence microscope. A + UroV required 4 double trisomic cells (chrom 3, 7, 17) or 12 cells with homozygous 9p21 deletion. Cases with UroV+ cells below threshold were considered inconclusive. We identified UC/UroV discordant cases, and reviewed UC slides for UC-/UroV+ cases. Minimum f/u was 18 mo.

Results:

Table 1

Category	# cases	Trisomy (# cases)	9p21 loss (# cases)	# cells counted (median)
UroV (-)/UC(-)	448	0	0	180
UroV(-)/UC(+)	64	0	0	63
UroV(+)/UC(+)	127	93	34	87
UroV(+)/UC(-)	12	11	1	19
UroV (inconclusive)/UC(+)	7	N/A	N/A	208
UroV (inconclusive)/UC(-)	9	N/A	N/A	147

N/A = not applicable

76 (11%) cases were discordant: UroV+/UC- (n=12; 2%); UroV-/ UC+ (n=;9%). For the UroV+/UC- cases, 3 had suboptimal UC (low cellularity/degeneration/obscuring). 10 cases had rare to many urothelial cells with high N:C ratios, but absent or mild atypia. 11 (92%) and 1 (8%) were trisomic and 9p21 deleted. Nine trisomic cases had ≥ 1 cell with concurrent chromosome 3/7/17 trisomy. Number of total cells counted to reach threshold number of UroV+ cells was 4-50 (median 19). Interval from UroV+ to + UC/bx was 1 - 210 days (median 32 days). Follow-up UN was high-grade in 8/12 (67%) cases; for UC+/UroV- cases, UN was high-grade in 26/64 (41%) cases.

Conclusions: 1) Discrepant UC/UroV occurred in 11% of cases. These cases were UC+/UroV-, suggesting lower UroV sensitivity for UN than UC. 2) UroV+ confers a high predictive value for UN in UC- cases. 3) Most UroV+/UC- pts are trisomic, similar to UroV+/UC+ pts. 4) Unlike UroV inconclusive pts, UroV+/UC- anticipatory (+) pts required only a small number of cells to be counted to reach threshold number of UroV+ cells. 5) Most UroV+/UC- anticipatory (+) pts have high-grade UN.

430 P16 Expression in Biopsies with Tubal Metaplasia from Patients with Atypical Glandular Cells on Pap Smear

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Background: A finding of atypical glandular cells (AGC) on pap smear is significant requiring further work-up. A proportion of these cases can be attributed to tubal metaplasia (TM), a known potential pitfall. P16 staining has been used as an adjunct in diagnosis and confirmation of significant squamous and endocervical dysplastic lesions. p16 immunostain can be positive in endocervical TM and this can be another potential pitfall when used in isolation to evaluate such lesions. The aim of this study is to evaluate p16 immunostaining in tissue specimens from patients who had TM accounting for AGC on their pap smears.

Design: Patients who had a diagnosis of AGC on their Thin-prep pap smears and with subsequent tissue diagnosis of endocervical TM were selected. Cases with coexisting squamous intraepithelial lesion (SIL) were excluded. Paraffin-embedded tissue of 16 cases including 9 cervical cone and 7 endocervical curettage (ECC) specimens were immunostained for p16 (clone 16PO4;1:300 dilution, Cell Marque Corp, Hotsprings, AZ). For comparison, 22 cases of endocervical AIS (13 cervical cones, 9 ECC) and 10 hysterectomy specimens with endometrial TM were also stained. Immunoreactivity was scored for both intensity (weak, intermediate and strong) and extent. Localization of staining (nuclear and/or cytoplasmic) was also noted.

Results: Cases with AGC on pap smear attributed to endocervical TM cells showed weak to strong p16 staining in 94% of cases with patchy staining confined to the TM cells giving a "mosaic appearance". In contrast, endocervical AIS showed intermediate to strong, diffuse uniform staining in the dysplastic endocervical epithelium. Staining was noted in both nucleus and cytoplasm in TM and AIS. Endometrial TM showed patchy mild to moderate p16 immunostaining in 90% of cases. Some endometrial glands without TM, normal and reactive endocervical cells may also show rare cells with weak staining that appears insignificant.

Conclusions: Significant p16 immunostaining may be seen in tissue biopsies with endocervical tubal metaplasia that presented as AGC on pap smear. Tubal metaplasia can mimic AIS both on pap smears and tissue specimens. A combination of morphologic features and patchy mosaic staining pattern with p16 are supportive of tubal metaplasia. There are recent articles suggesting utility of p16 immunostain in pap smears for diagnosis of SIL, and in our opinion this interpretation may need caution when evaluating cases presenting as AGC.

431 Trends in Thyroid Aspiration Biopsy over a Decade: Analysis of over 1400 Cases

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Background: In 2006, the American Thyroid Association produced guidelines for fine needle aspiration biopsy (FNAB) as the procedure of choice in the evaluation of thyroid nodules. However, recent published studies of thyroid FNAB by palpation or with ultrasound guidance show wide variability in non-diagnostic specimens and in "atypical or indeterminate" diagnoses, which often lead to surgery. We reviewed our experience with thyroid FNAB over a decade to assess trends in our practice.

Design: Annual (1995, 2000, and 2005) thyroid FNABs performed over eleven years at the NYU School of Medicine were reviewed and compared. The method of aspiration (by palpation or by ultrasound guidance) and the physician performing the aspiration (pathologist, radiologist, endocrinologist, or surgeon) were recorded. Diagnoses were classified as non-diagnostic, benign, atypical/suspicious (including those with mention of follicular and Hürthle cell neoplasms), or malignant. The majority of our diagnoses were made from direct smears.

Results:

table 1

	1995 (n=79)	2000 (n=480)	2005 (n=857)
METHOD OF ASPIRATION*			
Palpation	67 (85%)	134 (28%)	59 (7%)
Ultrasound Guidance	12 (15%)	346 (72%)	798 (93%)
DIAGNOSTIC CATEGORY			
Benign	61 (77.2%)	415 (86.5%)	781 (91.1%)
Malignant	5 (6.3%)	9 (1.9%)	22 (2.6%)
Non-diagnostic	6 (7.6%)	12 (2.5%)	17 (2.0%)
Atypical/Suspicious	7 (8.9%)	44 (9.2%)	37 (4.3%)

*In 1995, 52% of thyroid FNABs had on-site assessment by a pathologist compared to 69% in 2005.

Conclusions: 1) From 1995 to 2005 there was a ten-fold increase in the total number of thyroid FNABs. During this period, there was an increase in thyroid FNAB with ultrasound guidance from 15% to 93% and a corresponding decline by palpation. **2)** There was a progressive increase in the number of thyroid FNABs over eleven years, but the majority were benign accounting for the increase in the benign category from 77% to 91%. **3)** The number of malignant diagnoses was low and remained stable; ultrasound guidance did not adversely affect the rate of malignancy. **4)** Liquid based preparations were not necessary to achieve these results. **5)** There was a decrease in the

non-diagnostic category and the atypical category presumably due to more experience with the performance and interpretation of thyroid FNABs, increased on-site assessment, and increased ultrasound guidance.

432 Diagnostic Utility of Immunohistochemical Profile in the Classification of Pulmonary Non-Small Cell Carcinoma in the Fine Needle Aspiration Cytology Specimen

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Background: Fine needle aspiration (FNA) cytology is often used for the evaluation of lung cancers. The value of an accurate classification of pulmonary non-small cell carcinomas is becoming important in the era of EGFR-targeted therapies. Immunohistochemistry can be utilized in differentiating pulmonary adenocarcinoma (AC) from squamous cell carcinoma (SCCA) in resection specimens, but has not been systematically investigated in FNA specimens.

Design: Forty cases of lung FNA cytology specimens with cell blocks were retrieved from the hospital computer system. Cases consisted of 19 AC and 21 SCCA. All cases were confirmed by subsequent resection of the mass. The immunostaining for CK7, CK20, TTF-1, CK5/6, p63, and K903 was performed on cell blocks with an automated immunostainer using biotin-avidin-complex method with appropriate positive and negative controls.

Results: AC showed positivity for CK7 (78%), CK20 (26%), TTF-1 (79%), CK5/6 (0%), p63 (10%), and K903 (15%). SCCA exhibited positivity for CK7 (9%), CK20 (0%), TTF-1 (0%), CK5/6 (80%), p63 (66%), and K903 (95%).

Conclusions: CK5/6, p63 and K903 were significantly expressed in SCCA than AC, CK7 was observed in most AC and TTF-1 positivity was only seen in AC. Therefore, a panel of immunohistochemical studies including CK7, CK20, TTF-1, CK5/6, and K903 is useful in better classifying non-small cell carcinoma in FNA cytology specimens.

433 Cytology – A Potentially Useful Modality for Diagnosis of Ocular Lesions

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Background: Cytologic samples from the eye and its adnexa are a rarity in routine cytology practice. However accessibility and a wide diversity of lesions holds the potential for widespread utilization. In the present study we analyzed our archives for ocular cytology specimens to reflect on our diagnostic efficacy and potential utility of this modality.

Design: A retrospective review of our pathology database over the last ten years was performed to retrieve all cases of ocular cytology. Histologic correlates and clinical chart information were reviewed when available.

Results: There were a total of 24 specimens from16 patients including three Fine Needle Aspirations (FNA), five vitreous fluids (VF) and sixteen corneal/ conjunctival brush or scrapes (CB). The three FNA samples with diagnoses of lymphoma/ leukemia had histologic correlates reiterating the cytologic diagnoses. Of the five VF samples (three patients), one was positive for lymphoma, three were negative and one unsatisfactory. VF analysis had been predominantly performed for diagnosis and follow up of lymphoma. The sixteen CB samples from 11 patients had the following diagnoses rendered - negative cytology (9), mild dysplasia/ atypical squames (4) and severe dysplasia (3). Biopsy diagnosis available in the one patient with negative cytology was amyloid keratopathy. Samples from other patients with negative cytology had been submitted with clinical diagnoses of possible ocular surface neoplasia, viral infections, keratoconjunctivitis, evaluation of ocular surface extension of a neoplasm in the vicinity or follow-up of patients with known ocular surface dysplasia. Of the four specimens (three patients)with atypical squames/ mild dysplasia, one had corresponding histology and the other two were clinically followed up with therapeutic interventions. The three specimens (two patients) with severe dysplasia had histologic diagnoses of severe corneal intraepithelial neoplasia and squamous cell carcinoma. Thus FNA cytology had 100% concordance with histology and in no instance did ocular surface cytology miss dysplasia.

Conclusions: Our experience highlights the efficacy of cytology for diagnosis of ocular lesions, especially so for ocular surface dysplasia and explores its role as a potentially useful technique in routine practice. We feel that increasing awareness of this modality among pathologists and ophthalmologists might make it a widely accepted technique for triaging patients with accessible ocular lesions.

434 A Comparison of Cytology and Fluorescence *In Situ* Hybridization for the Detection of Malignant Bronchial Brushing and Washing Specimens

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Background: The routine bronchial brushing and washing cytology is an important diagnostic tool in the evaluation of patients with suspected lung cancer. Fluorescence in situ hybridization (FISH) is a valuable tool to detect chromosomal abnormalities. FISH has been shown to yield superior sensitivity over routine cytology for the detection of urothelial carcinoma. The LAVysion FISH probes (Abbott Laboratories) are developed for the detection of lung cancer, and contain locus-specific probes to 5p15, 7p12 (EGFR), 8q24 (c-Myc), and a centromeric probes to chromosome 6. The purpose of this prospective study is to evaluate the performance of LAVysion FISH assay over routine cytology for the detection of malignant bronchial brushing and washing specimens.

Design: Forty six consecutive patients who underwent bronchoscopic examination in a period of 6 months were included in the study if bronchial brushing and / or washing specimens were accompanied by concurrent endobronchial biopsy and / or transbronchial fine needle aspirations (FNA), which were used as gold standard. The smears for routine cytology were prepared first using Thin Prep technology and direct smear. The smears for FISH were made from the residue using cytospin preparation. The FISH analysis utilized the commercially available LAVysion probes. The FISH was reported positive if $\geq$ 6 cells with gains of two or more chromosomes in the same cell, or $\geq$ 10 cells with all four chromosome copy numbers at 4N. The indeterminate cytology results were considered as negative for statistical analysis.

Results: Twenty eight of the 46 patients were diagnosed of malignancy (22 non small cell carcinoma, 5 small cell carcinoma and 1 granular cell tumor) based on biopsy and / or FNA. For bronchial brushing specimens, the sensitivity of routine cytology and FISH for the detection of malignancy was 17% (3/18) and 33% (6/18) respectively (p=0.08); the specificity of routine cytology and FISH was the same, 78% (7/9). For bronchial washing specimens, the sensitivity of routine cytology and FISH for the detection of malignancy was 8% (2/25) and 28% (7/25) respectively (p=0.025); the specificity of routine cytology and FISH was the same, 94% (15/16).

Conclusions: Our data shows that bronchial cytology appears to have a very low sensitivity for the detection of malignancy and FISH analysis does not appear to improve it to a desirable level. A larger study is necessary to further evaluate the role of FISH analysis in the detection of malignancy on bronchial cytology specimens.

435 A Comparison of Cytology and Fluorescence *In Situ* Hybridization for the Detection of Malignant Bile Duct Brushing Specimens

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Background: The routine bile duct brushing cytology is an important diagnostic tool in the evaluation of patients with bile duct stricture. The routine cytology has high specificity but rather poor sensitivity for the detection of malignancy. The leading causes of malignant biliary tract stricture are cholangiocarcinoma and pancreatic adenocarcinoma, which are characterized by high frequency of numerical and structural chromosomal abnormalities. Fluorescence in situ hybridization (FISH) is a valuable tool to detect chromosomal abnormalities. The UroVysion FISH probes (Abbott Laboratories) are originally developed for the detection of urothelial cancer, and contain probes to the cnetromeres of chromosome 3, 7 and 17, and chromosomal band 9p21. The purpose of this prospective study is to evaluate the performance of UroVysion FISH assay over routine cytology for the detection of malignant bile duct brushing specimens.

Design: Thirty five consecutive patients who underwent ERCP and bile duct brushing for bile duct stricture in a period of 6 months were included in the study. The smears for routine cytology were prepared first using Thin Prep technology. The smears for FISH were made from the residue using cytospin preparation. The FISH analysis utilized the commercially available UroVysion probes. The FISH was reported positive if $\geq$ 4 cells showed gains for $\geq$2 chromosomes. The indeterminate cytology results were considered as negative for statistical analysis.

Results: Twenty two of 35 patients were diagnosed of malignancy (22 adenocaricnoma and 1 multiple myeloma) based on biopsy, fine needle aspiration or clinical progression of disease. The sensitivity of routine cytology and FISH for the detection of malignancy was 14% (3/22) and 55% (12/22) respectively (p=0.003). The specificity of routine cytology and FISH was 100% (13/13) and 62% (8/13) respectively (p=0.025).

Conclusions: Our study shows that FISH is significantly more sensitive than routine cytology for the detection of malignancy in bile duct brushing specimens. However, the specificity of FISH is extremely poor in our study, compared to routine cytology with excellent specificity. A larger study is necessary to further evaluate the performance of FISH in the detection of malignancy in bile duct brushing specimens.

436 Follow-Up Outcomes of Cytological and Histological Abnormalities among Women with Negative Computer-Imaged Liquid-Based Pap and Positive HPV DNA Test Results

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Background: Limited data from the U.S. and overseas have been reported on the natural history of hrHPV positive women screened with negative conventional Pap smear results, but no reports have documented follow-up of cytology negative hrHPV positive women routinely screened with liquid-based cytology (LBC), computer-assisted screening, and HPV co-testing. The purpose of this study was to document the development of cytological and histological abnormalities among women who tested hrHPV positive along with negative Pap testing.

Design: The hospital records of MWH were searched for patients reported as negative on ThinPrep Imaging System-imaged ThinPrep Pap tests (TPPT) who also had positive HC2 hrHPV with co-test results between July 2005 and December 2007. Cytologic and histologic follow-up outcomes were analyzed.

Results: During the study period 402 women with negative TPPT and concurrent positive hrHPV results had documented cytologic and/or histologic follow-up. Histologic follow-up included 111 women who underwent cervical biopsy with or without ECC and 39 who underwent ECC alone. The mean age was 41.6 years (15-84 years). The average follow-up period was 13 months, ranging from 1 to 35 months (mean 10.6 m). Follow-up results documented that 8 of 402 (2.0%) women had tissue diagnoses of intraepithelial neoplasia 2+, including, four CIN2, two CIN3, one VAIN3, and one case of AIS with microinvasion. CIN1 was detected in 61 women(15.2%). 82 (20.4%) had follow-up ASC-US Pap test results. All CIN 2+ and 50 of 61 CIN 1 lesions were diagnosed based on histology. The interval between positive HPV/negative TPPT and diagnosis of CIN 2+ ranged from 1 month to 19 months (median 14 months).

Conclusions: CIN3, often proposed as a surrogate for invasive cervical cancer in cervical screening trials, was detected in 2 of 402 women (0.5%) with negative TPPT and concurrent positive HPV. Inclusion of two additional cases of histologically detected CIN2, one VAIN3, and one case of AIS with microinvasive endocervical adenocarcinoma potentially alters the risk profile of this cohort of over 400 cytology negative HPV positive patients. Additional natural history studies are needed on cytology negative HPV positive women routinely screened with modern methods which are now prevalent in the U.S. The potential benefits of routinely combining LBC and HPV co-testing for enhanced detection of endocervical neoplasia deserves special study, given the limited reported success of screening in this area to date.