Cytopathology

267 Efficient Cell Blocks Appear Improve Diagnosis of Ultrasound-Guided FNAs of Non-Cystic Breast Lesions without a Need for On-Site Adequacy Evaluation

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Background: Cell blocks provide diagnostic architectural information that complements smears or monolayers. On-site evaluation (OSE) of breast FNAs makes the on-site assessed material unavailable for a cell block. We previously found that a new "rapid cell block" technique is highly efficient at recovering sparse material, and improves diagnosis by frequently permitting diagnosis of invasion in FNAs of breast cancers or allowing accurate histologic grading of proliferative lesions. We tested the hypothesis that one monolayer slide and a rapid cell block without OSE would outperform OSE with multiple smears and a conventional cell block for ultrasound-guided breast FNAs.

Design: Due to relocation of Pathology in October 2005, we stopped performing OSE for breast FNAs. Instead, the same mammographers performed two passes, with additional passes if needle rinses showed no particles in the needle rinse. Rapid cell blocks were made after a ThinPrep®. We examined the outcome of 134 consecutive non-cyst FNAs without OSE from October 2005 to April 2006, and compared them to 119 consecutive non-cyst FNAs from 2004 that had OSE and a conventional (collodion bag) cell block only when there was a visible pellet after centrifugation.

Results: In the OSE cohort, 1.4 OSEs were performed per case, and sufficient material remained in 25% for a cell block, though with generally sparse cellularity. 13% of OSE FNAs were "Unsatisfactory for diagnosis" compared to 11% in the cohort without OSE. The rates of "gray-zone" diagnoses (atypical plus suspicious) were 19% with OSE and 11% without OSE ($p< .1$). 6/19 atypical FNAs with OSE had cancer at follow-up compared to only 1/13 atypical FNA without OSE (a case without a rapid cell block). Neither cohort had false positive diagnoses, or cancers if the FNA was negative. Time per OSE was estimated at 25 minutes for pathologists and 15 minutes (extra) for mammographers for a savings of 35 and 21 minutes per case, respectively. At Medicare reimbursement rates for OSE and cell blocks, the cost per patient was reduced by $48.

Conclusions: Our results suggest that replacing OSE with efficient cell blocks can provide more clear-cut results, while saving time for pathologists and clinicians, and saving health care dollars.

268 Evaluation of Diagnostic Efficacy of Image Guided Fine Needle Aspiration Biopsy of Neoplastic and Non-Neoplastic Lung Lesions: Retrospective Study of 721 Cases

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Background: Image guided fine needle aspiration biopsy (FNAB) has become a favored diagnostic method for evaluating benign and malignant lesions of the lung. To determine the diagnostic efficacy of this procedure, a 10-year retrospective study of 721 FNAB's of pulmonary lesions performed at our institution was undertaken.

Design: Image guided FNAB of lung performed during a 10-year interval, from January1996 to February 2006 were retrieved from the cytology archives. There were 721 specimens from 665 patients, 399 males and 266 females. Their ages ranged from 5 to 93 years (mean age~ 49 years). Concurrent core needle biopsy specimen or surgical resection material for follow up was available for 523 patients. The remaining 142 patients who had no relevant tissue available were followed by review of their medical records.

Results: The FNAB results were divided into three categories and these were: 1.Positive and suspicious for malignancy. 2. Benign lesions including benign neoplasms, infectious or inflammatory conditions. 3. Negative for malignancy. Based on these results, 383 cases (53.1%) were in the malignant category, 59 cases (8.2%) were benign and 279 cases (38.7%) were negative. The positive for malignancy category consisted of 301 cases of primary lung cancer, 68 cases of metastastic malignancies and 14 cases were ultimately considered false positive. The most common primary malignancy was non-small cell carcinoma (89 cases), followed by squamous cell carcinoma (83 cases), adenocarcinoma (79 cases) small cell carcinoma (32 cases), carcinoid (5 cases) and others (13 cases). Out of the 338 FNAB's that were categorized as benign diagnoses (including benign neoplasms, infectious or inflammatory lesions) and negative conditions, 40 cases were false negative.

Conclusions: This study, to the best of our knowledge is one of the largest series of pulmonary lesions diagnosed by FNAB. It showed an overall sensitivity of 90.22% and specificity of 95.51 %. The positive and the negative predictive values were 96.34% and 88.16%, respectively. From our experience, we conclude that fine needle aspiration biopsy of intrapulmonary lesions is highly sensitive and specific technique for diagnosis of both neoplastic and non-neoplastic pulmonary lesions. It is one of the best initial diagnostic tools for triaging radiographically detected intrapulmonary lesions.

269 The Contribution of Fine Needle Aspiration to the Diagnosis of Image-Guided Biopsies of Non-Hodgkin's Lymphoma

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Background: Deep seated lesions suspected to be non-Hodgkin's lymphoma typically undergo image-guide needle sampling in order to obtain material for pathologic examination. The purpose of this study was to investigate the contributions of fine needle aspiration (FNA) and core biopsy (CB) in simultaneously sampled lesions in the diagnosis of non-Hodgkin's lymphoma (NHL).

Design: A retrospective review was conducted of image-guided biopsies of NHL obtained over a 44 month period when concurrent FNA and CB sampling had been performed. Patients underwent FNA with on-site assessment of adequacy followed by 14-18 G CB. The FNA and CB were evaluated and reported independently with the FNA blinded to the results of the CB. The final diagnoses were categorized as unsatisfactory, suspicious for NHL, NHL with incomplete classification or NHL with definitive categorization.

Results: 99 cases were identified from 97 patients with a mean age of 57.8 years (M:F 1.4:1). When combined, CB and FNA achieve the highest rate of definitive classification with 80 cases (80.8%) NHL with definitive categorization, 10.1% NHL with incomplete diagnostic classification, 5.1% suspicious and 4.0% unsatisfactory samples. FNA provided a definitive categorization in 10 cases (10.1%) when the CB was either unsatisfactory, suspicious, or NHL with incomplete classification. CB alone achieved 70.7% NHL with definitive categorization, 11.1% NHL with incomplete classification, 5.1% suspicious and 13.1% unsatisfactory samples. FNA alone accomplished 55.6% NHL with definitive categorization, 20.2% NHL with incomplete classification, 11.1% suspicious for NHL and 13.1% unsatisfactory samples. The diminished independent performance of FNA was largely due to inadequate sample collection. When only FNA samples deemed adequate from on-site assessment were analyzed, the diagnostic rates for CB and FNA equalized. FNA samples adequate at on-site evaluation (69 cases) achieved 72.5% NHL with definitive categorization, 17.4% NHL with incomplete classification, 8.7% suspicious and 1.5% unsatisfactory samples.

Conclusions: The combination of FNA with CB provides the greatest diagnostic yield in image-guided sampling of NHL with FNA providing definitive categorization on some samples when CB was either unsatisfactory, suspicious, or NHL with incomplete classification. However, adequate sample collection is paramount to achieving definitive classification of NHL by FNA.

270 Concordance of Immunophenotype Obtained by Laser Scanning Cytometry and Flow Cytometry of Lymphoid Lesions and Lymphoma in Cytologic Samples

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Background: Determination of the immunophenotype is a significant component in the evaluation of lymphoid lesions and is often achieved using flow cytometry (FC). However, the need for large quantities of cells may limit application of FC to cytologic samples. Laser scanning cytometry (LSC) is a novel method to immunophenotype similar to FC, but utilizes a glass slide-based preparation consuming far less sample, making it ideally suited for cytologic specimens. This study was initiated to compare the immunophenotype obtained by LSC of cytologic samples of lymphoid lesions to FC.

Design: Cases from a 26 month period were retrospectively identified for the study if there had been successful immunophenotyping by LSC and either simultaneous FC immunophenotyping or if FC had been obtained from a different biopsy, but within a 90 day period either preceding or following the sample for LSC. Concordance of the immunoprofiles was determined for the population and for the specific markers; Kappa, Lambda, CD19, CD20, CD10, CD23, FMC7, CD11c, CD2, CD3, CD5, CD4, CD7, CD8. In cases in which discordances were present, the LSC and FC results were reviewed in a blinded manner.

Results: 72 samples from 68 patients were identified consisting of 65 fine needle aspirations, 6 body cavity fluids and 1 bronchoalveolar lavage. In 23 cases, simultaneous LSC and FC immunophenotyping was performed. In 49 cases, FC immunophenotyping was obtained either preceding (6 cases) or following (43 cases) the sample immunophenotyped by LSC. There was a high degree of concordance of the immunophenotypes from LSC and FC. In the 23 cases with concurrent LSC and FC, no discordances in immunophenotype were found that would result in changes in diagnostic classification, although one or more marker showed minor discordances in 6 cases; most commonly FMC7. In the cases in which FC was performed on a sample different from that for LSC, 12 of 49 cases showed minor discordances in FMC7, CD11c and CD23 that would not alter the diagnostic classification of the lesions. In 1 case, LSC did not identified CD10 expression found by FC and in 1 case FC did not detect light chain clonality revealed by LSC.

Conclusions: The immunophenotype obtained by LSC on cytologic samples shows concordance with that obtained by FC and generates results that are diagnostically equivalent. Non-correlating markers appear to be the result of differences in antibodies used, non-specific antibody binding, sampling and viability issues.

271 Human Papillomavirus (HPV) Genotyping and Biopsy Correlates of Low Grade Squamous Intraepithelial Lesion, Can Not Exclude a High Grade Lesion (LSIL-H): A Comparison with ASC-H, LSIL and HSIL

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Background: The diagnosis of LSIL-H was not included in the Bethesda 2001 (B2001) classification but is used in some institutions to diagnose cases showing a few cells that are suspicious but not diagnostic for HSIL in addition to changes diagnostic of LSIL. Such cases fulfill the B2001 criteria for both the diagnosis of LSIL and ASC-H. This study reviews our experience with cases diagnosed as LSIL-H, a diagnostic category that we have recently introduced into our practice.

Design: The computerized records of our institution were searched for cases diagnosed as LSIL-H, LSIL, ASC-H and HSIL during the period from 01/01/2004 (the date we started using the diagnosis of LSIL-H) to 6/30/2006. Clinical data, PCR-based HPV test results and biopsy follow-up data were collected on all cases. The Pap tests (PT) and biopsies were diagnosed by 15 academic and community pathologists; no review of the slides was undertaken. PCR-based HPV testing was performed on the residual liquid-based PT sample using the MY09/11 consensus primers and typing was performed by Restriction Fragment Length Polymorphism (RFLP).

Results: During the interval of the study our laboratory processed 150,962 PT (97% Surepath, 1.5% Thinprep and 1.5% conventional smears). The diagnoses of LSIL-H, ASC-H, LSIL and HSIL were made in 0.23%, 0.56%, 1.97%, and 0.39% respectively. Biopsy follow-up was available in 164/350 LSIL-H (47%), 1598/2970 LSIL (54%), 507/840 ASC-H (60%) and 485/652 HSIL (74%) cases. Valid HPV results were available for 24 LSIL-H, 200 LSIL, 349 ASC-H and 36 HSIL cases.

	PCR HPV TESTED	% HPV+ ALL TYPES	% HR-HPV+ OF ALL HPV+	% HPV16+ OF ALL HPV+	BIOPSY F-U	BIOPSY CIN2/3+
LSIL-H	24	75%	67%	39%	164	31%
LSIL	200	79%	46%	18%	1598	17%
ASC-H	349	49%	73%	41%	507	27%
HSIL	36	81%	76%	45%	485	66%

Conclusions: LSIL-H is a rare diagnosis, comprising only 0.23% of the 150,962 PT evaluated in our institution during the study period. Follow-up biopsy data show that the CIN2/3+ rates after a diagnosis of LSIL-H (31%) are similar to those of ASC-H (27%) and significantly higher than those of LSIL (17%). This is also reflected in the similar distributions of HPV types of LSIL-H and ASC-H. Our results support the addition of LSIL-H to the diagnostic categories proposed by B2001 and suggest that the management of women with this diagnosis should be similar to that of women with ASC-H.

272 Utility of UroVysion Testing in the Setting of Atypical Urine Cytology

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Background: Atypical urine cytology raises a broad differential diagnosis ranging from benign reactive changes to low grade and high grade urothelial carcinoma (UC). Fluorescence in-situ hybridization (FISH) by the UroVysion system provides an ancillary test for screening urine samples for urothelial carcinoma (UC). The test is targeted to negative and atypical urine specimens and identifies molecular cytogenetic changes characteristic of UC. We reviewed our experience to date with UroVysion testing in cytologically atypical urine samples.

Design: We conducted a retrospective review of UroVysion results on all urine samples in which a diagnosis of "atypical" was rendered by routine cytologic evaluation. UroVysion testing commenced on 9/21/2004 and we reviewed cases up to 7/20/2006. Cytology review and UroVysion were requested by the clinician for surveillance and detection of recurrent UC.

Results: UroVysion testing was performed on a total of 176 cases with a cytologic diagnosis of "atypical". UroVysion testing was negative in 135 (77%) cases, positive in 25 (14%) cases, inconclusive in 5 (3%) cases and insufficient in 11 (6%) cases. Follow-up surgical pathology was available in 15 (60%) of the 25 UroVysion positive cases: 4 cases showed high grade UC, 4 cases showed low grade UC, 2 cases showed dysplasia, 2 cases were atypical and 3 cases were benign.

Conclusions: UroVysion testing was positive in a small but significant number (14%) of cytologically atypical urine samples, indicating the presence of neoplastic changes not detected by routine cytologic evaluation. Although follow up was limited, only a small percentage of the cases with follow up were found to have high grade UC. Notably, a large percentage of cases (77%) did not show UroVysion abnormalities. The utility of UroVysion testing may be limited and targeted application to specific cases may be useful.

273 Serous Cystadenomas of the Pancreas: Diagnostic Performance of Imaging and Fine Needle Aspiration Biopsy

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Background: Expectant management for serous cystadenomas (SCA) of the pancreas requires an accurate pre-operative diagnosis. Current radiological criteria for SCAs, particularly the oligocystic variant, are not reliable. Prior published cytologic diagnostic sensitivities have ranged widely from 10% to a 100%. In this study we evaluate the diagnostic sensitivity of endoscopic ultrasound (EUS)-guided fine needle aspiration biopsy (FNAB) and cross sectional imaging for SCAs.

Design: Group I consisted of 22 histologically confirmed SCAs. Group II (n=8) lacked histologic confirmation and was defined by EUS findings that were consistent with a SCA and cyst fluid CEA <5 ng/ml. Cross sectional imaging data was recorded. Twenty-two aspirates were EUS-guided, two were CT-guided and six were obtained intraoperatively. The smears were evaluated for the presence of serous lining epithelium, gastrointestinal contaminating epithelium and inflammatory cells including hemosiderin-laden macrophages. We also evaluated the presence of hemosiderin-laden macrophages in a series of 110 fine needle aspirates from histologically confirmed mucinous tumors of the pancreas.

Results: Cross sectional imaging studies on group I cases prospectively provided a definitive diagnosis in 4 of 14 cases (29%). The histologically-confirmed SCAs had CEA levels of less than 5 ng/mL, except one which was 176.5 ng/mL. On FNAB, 18 cases (81%) were classified as negative and 4 (19%) as atypical. Among these, a cytologic diagnosis of SCA was made prospectively in only one CT-guided case. Retrospectively, three intraoperative aspirates and one CT-guided aspirate contained rare epithelial cells of a SCA. None of the EUS-guided aspirates demonstrated serous epithelium. Among group II aspirates, only one contained serous epithelial cells. Fifty-nine percent of the EUS-guided aspirates showed gastrointestinal contamination. This epithelium was catagorized as 'atypical' in 4 cases. Macrophages were identified in 19 (63%) cases, 12 (40%) of which contained hemosiderin. Conversely, only 3 of the 110 (3%) mucinous tumors of the pancreas showed hemosiderin-laden macrophages.

Conclusions: The sensitivity of cytology for SCA is poor with only 20% of cases demonstrating serous lining epithelium. Gastrointestinal contaminating epithelium, often seen in EUS-guided aspirates, further contributes to difficulties in interpretation. The presence of hemosiderin-laden macrophages as a surrogate marker for SCA requires further study.

274 Scraped Cell Block Technique in Fine Needle Aspiration: A Review of Five Years' Experience

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Background: Cytologic examination of cellular material obtained via fine needle aspiration (FNA) techniques is common; however, in many cases the aspiration specimen does not yield sufficient information for a precise diagnosis. In these cases, the application of immunostaining procedures can be of diagnostic importance. Some cytologic specimens are limited in quantity, which may hamper or preclude the performance of immunocytochemistry if more than one antibody is required for a definitive diagnosis. In these instances, scraping previously fixed and stained cytologic preparations to create a paraffin-embedded cell block allows for the application of multiple antibodies. While there are several published methods for assessing FNA specimens with immunocytochemistry, there is little published regarding the utility of these techniques.

Design: The objective of this study was to assess the utility of immunocytochemical studies performed on a "scrape cell block," using a variety of antibodies. Cytology specimens received during a five-year period in which a scrape cell block was used for diagnostic purposes were retrieved from archives, reviewed, and compared with histological diagnoses.

Results: A total of 47 cases were studied. In 39 cases (83%), the immunocytochemistry performed on the scrape cell block yielded useful information. The immunocytochemistry data obtained from the scrape cell block established the diagnosis in 19 cases (40%), refined the diagnosis in 17 cases (36%), and supported the diagnosis in 3 cases (6%). In 3 cases (6%) no additional information was provided. In 5 cases (11%), the scrape cell block was technically unsatisfactory.

Conclusions: Immunocytochemistry performed on cytologic preparations is a valuable diagnostic tool. The use of scrape cell blocks permits the application of multiple antibodies and allows for more precise diagnoses on limited cytologic samples.

275 The Value of Cytogenetics as an Adjunct to Cytology in the Diagnosis of Non-Hematopoietic Neoplasms

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Background: Molecular techniques are gaining wider acceptance in diagnostic cytology. This study explores the diagnostic utility of karyotypic analysis or FISH on FNAs of non- hematopoietic neoplasms.

Design: We performed a retrospective analysis of FNAs from January 1999 to June 2006 in which a portion of the aspirated material was sent for karyotype or FISH.

Results: Analysis was successful in 63 (36%) of 175 FNAs sent for conventional cytogenetic (karyotypic) or FISH analysis. Diagnostically useful information was obtained in 32 cases (18%). A karyotype was successful in 34 cases, with a clinically relevant result in 10. Characteristic chromosomal abnormalities specific for subtypes of renal cell carcinoma (RCC) were found in 5 cases (trisomies 7, 12, 16, 17, and/or 20 in 4 papillary carcinomas and loss of 3p in one clear cell carcinoma). One case showed t(9;22), a characteristic finding in extraskeletal myxoid chondrosarcoma. Trisomies 5 and 7 were found in a soft tissue mass supporting the morphologic impression of an extraarticular diffuse type giant cell tumor. In one patient with widely metastatic disease, an FNA of a pelvic mass showing poorly differentiated carcinoma revealed isochrome 1q, which suggested an endometrial primary site. A normal karyotype was found in 20 cases. It was helpful in 2 cases (to exclude metastatic RCC and myxoid liposarcoma). Nonspecific abnormalities were found in 4 cases. Cultures failed in 113 cases, nearly all due to sparse cellularity. FISH was performed on 31 FNAs, providing diagnostically useful information in 22 cases. An EWS rearrangement was found in 6 Ewings/PNETs, 1 desmoplastic small round cell tumor, and 1 clear cell sarcoma. Two cases showed rearrangement of the 18q11 SYT gene, a characteristic finding in synovial sarcoma. Trisomies 7 and 17 were detected in 3 cases of papillary RCC. Multiple copies of chromosome 12 confirmed the cytologic impression of a malignant germ cell tumor. A negative FISH result was helpful in 8 cases: to exclude Ewings/PNET (3 cases), synovial sarcoma (2 cases), both Ewings/PNET and synovial sarcoma (1 case), and papillary RCC (2 cases).

Conclusions: Informative cytogenetic results can be obtained from FNAs, most often from tumors with well-characterized chromosomal aberrations such as many soft tissue tumors and RCCs. When used selectively, cytogenetics can play a valuable role as an adjunct to diagnostic cytology.

276 Liquid-Based Pap Tests Interpreted as ASC-US in the Presence of Candida and/or Bacterial Vaginosis: Correlation with HPV DNA Status, Cervical Biopsy Results, and Patient Age

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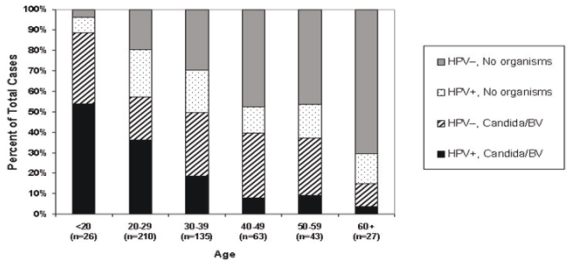
Background: Previous studies have suggested a relationship between HPV and the presence of Candida and bacterial vaginosis (BV) on liquid-based Pap tests (LBPT). This association is intriguing since HPV is sexually transmitted while Candida and BV are not. The purpose of our study was to compare the rate of HPV infection and follow-up cervical biopsy results in cases of ASC-US with and without Candida/BV.

Design: LBPT (SurePath, TriPath Imaging) diagnosed as ASC-US that underwent HPV DNA testing (Hybrid Capture 2, Digene) between June 2002 and August 2006 were identified. Patient age, results of HPV DNA tests, and follow-up biopsy results for all cases with Candida (n=122), BV (n=119), or both (n=14) were recorded and compared to a temporally matched control group of ASC-US cases that lacked organisms (n=249). Statistical analyses were performed using Chi-square and logistic regression.

Results: 49.0% of cases with Candida and/or BV were HPV positive compared with 39.4% of cases with no organisms ($p<0.05$). The majority of the HPV positive cases were in younger patients, as were most cases of Candida/BV (Fig 1). When adjusted for age, the difference in HPV positivity between cases with and without organisms was no

longer significant (p=0.67). Of the cases that had a follow-up biopsy, 56.6% (30/53) of cases with organisms showed SIL compared to 38.2% (21/55) of cases with no organisms (p=0.06). When adjusted for age, this difference remained not significant (p=0.12).

Fig 1. Correlation Between HPV Status, Presence of Candida/BV, and Patient Age



Conclusions: Our data support a correlation between HPV infection and Candida/BV, as described in previous studies. However, when adjusted for age, the rate of HPV infection in patients with and without Candida/BV was similar in our study. This likely reflects the increased prevalence of both Candida/BV and HPV infection in younger patients, rather than an increased susceptibility to HPV infection in the presence of Candida/BV. The finding that a significant proportion of young patients with Candida/BV are HPV positive and have SIL on follow-up biopsy suggests that cytopathologists should not adjust their threshold for diagnosing ASCUS/LSIL when Candida or BV are present.

277 The Diagnostic Utility of Cytology of Pancreatic Lesions in the Intraoperative Consultation

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Background: Cytology is an important diagnostic tool that is routinely used alone or accompanied by a frozen section (FS) during intraoperative consultation (IOC) at our institution. The purpose of this study is to compare the diagnostic accuracy of IOC cytology alone with that of cytology and FS together to evaluate pancreatic specimens.

Design: The institutional database was searched from August 2002 to August 2006 to identify pancreatic specimens with neoplastic and non-neoplastic lesions evaluated during IOC. The cases were separated into those evaluated by cytology alone and those evaluated by cytology and frozen section combined. *True positives* and *true negatives* were defined as interpretations that were in agreement with the final diagnosis. *False positives* and *false negatives* were defined as interpretations that were not in agreement with the final diagnosis.

Results: Seventy-five cases were identified. Thirty-nine (52%) cases were evaluated by cytology alone and 36 (48%) were evaluated by both cytology and frozen section. The final diagnosis was established by histologic diagnosis obtained following surgery. Of the 39 cases evaluated by cytology alone, 37 (95%) show agreement with the final diagnosis. Agreement was reached in 32 (89%) of 36 cases evaluated by both cytology and frozen section. The sensitivity of IOC cytology alone was 100%; the specificity was 92%. The sensitivity of IOC cytology and frozen section combined was 77%, and the specificity was 96%.

	True +	True -	False +	False -	Sensitivity (%)	Specificity (%)
IOC Cytology n=39	15	22	2	0	100	92
IOC Cytology+FS n=36	10	22	1	3	77	96

Conclusions: The study showed a higher sensitivity and comparable specificity for IOC cytology alone in the evaluation of pancreatic specimens in comparison to IOC cytology and frozen section combined. In experienced hands, cytologic evaluation during intraoperative consultation of pancreatic specimens is as highly accurate a diagnostic tool as frozen section while offering the advantages of decreased turn around time and preservation of the tissue for permanent section.

278 Should Negative Pap Tests Be Reviewed in Patients with a Newly Diagnosed Endometrial, Cervical or Vaginal Adenocarcinoma?

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Background: Cervicovaginal cytology is primarily a screening test for squamous intraepithelial lesions and squamous cell carcinoma. The detection of glandular lesions in Pap tests has been less successful due to a low sensitivity. The main contributing factors include sampling problem as well as interpretation. Our aim was to determine if retrospective review of negative Pap tests has a value in helping detect glandular epithelial lesions that have been missed in the initial evaluation.

Design: A total of 151 cases with a newly diagnosed endometrial (137 cases), cervical (3 cases), or vaginal (11 cases) adenocarcinoma were retrieved from 1998 to 2004. Their negative Pap tests, performed in the prior 6 months, were reviewed by cytopathologists who were blinded to the original interpretation. The cytopathologists re-interpreted the Pap tests and classified the glandular abnormalities using the Bethesda 2001 terminology, including atypical glandular cells (AGC), atypical glandular cell favor neoplastic (AGC-N), and adenocarcinoma (ACA).

Results: In the retrospective review of the 151 cervicovaginal smears, glandular epithelial abnormalities were found in 37 smears (24.5%). These abnormalities included 27, 5, 5 cases of AGC, AGC-N, and ACA, respectively. In 34 cases with endometrial adenocarcinoma, the Pap tests of 31 cases had newly recognized glandular epithelial abnormalities including 2 cases in which benign endometrial cells were mentioned in the original report. Three cases (2%) were upgraded from AGC to AGC-N or ACA. Regarding the histologic grades of endometrial adenocarcinoma, the glandular epithelial abnormalities were found in 21 of 80 (26%) well differentiated, 7 of 25 (28%) moderately differentiated, and 4 of 17 (24%) poorly differentiated adenocarcinomas.

Conclusions: Retrospective review of negative Pap tests in patients with a newly diagnosed endometrial, cervical and vaginal adenocarcinoma shows a significant number of glandular epithelial abnormalities. These findings indicate that interpretation error plays a role in Pap test false negative diagnoses of glandular cell abnormalities and further study of these lesions could improve detection.

279 Endoscopic Ultrasound-Guided Fine Needle Aspiration of the Liver

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Background: Endoscopic ultrasound-guided fine needle aspiration (EUS-guided FNA) has proved itself to be a valuable tool for the diagnosis and staging of pancreatic, upper gastrointestinal (GI), and pulmonary malignancy. It allows for the sampling of primary lesions and possible metastases during the same procedure thus decreasing the potential number of diagnostic procedures patients may have to undergo. Although a number of papers have addressed its efficacy with the sampling of nodal disease, papers evaluating its use with liver lesions have been limited by relatively small numbers of cases. We investigated our experience with EUS-guided FNA of the liver and compared it with other modalities used at our institution.

Design: Our cytology database was searched for all liver FNA from over a 6.5 year period. All liver FNA had been performed by radiologists or gastroenterologists with the assistance of on-site interpretation of adequacy and specimen triaging by a pathologist. The image-guided modalities used as well as the final diagnoses were recorded and compared.

Results: From 1/1/00 to 6/30/06, there were 282 FNAs of the liver of which 194 (69%) were EUS-guided FNA. Final diagnoses included metastatis (158; 56%), primary hepatic malignancy (24; 9%), benign process (80; 28%), and non-diagnostic specimen (17; 6%). EUS-guided FNA was more likely to sample metastatic pancreatic and upper GI malignancies ($p<0.05$) and benign processes ($p<0.05$) and was less likely to sample primary hepatic malignancy ($p<0.05$).

Table 1: EUS-Guided FNA vs CT/US-Guided FNA

Diagnosis	EUS FNA	CT/US FNA	Significance
Metastasis	109 (56%)	49 (56%)	NS
Metastatic Pancreas / Upper GI	34 (18%)	5 (6%)	p<0.05
Primary Hepatic Malignancy	9 (5%)	15 (17%)	p<0.05
Benign Process	62 (32%)	18 (20%)	p<0.05
Non-diagnostic	13 (7%)	4 (5%)	NS

NS: No Significance

Conclusions: EUS-Guided FNA is more likely than CT or routine ultrasound-guided FNA to sample metastatic pancreatic or upper GI malignancy and less likely to sample primary hepatic malignancy, consistent with the current work-up practices at our institution. It has a comparable non-diagnostic rate when compared to other modalities. The increased sampling of benign processes suggests either less discriminating use or, potentially, a sampling issue.

280 Detection of EGFR Gene Mutations in Archival Cytology Specimens by High Resolution Melting Analysis of PCR Amplicons

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Background: Mutations in epidermal growth factor receptor (EGFR) characterize a subset of lung carcinomas. Identifying these mutations is important because treatment with tyrosine kinase inhibitors (TKI) such as gefitinib and erlotinib has been correlated with clinical outcome. Currently, mutations are identified by PCR and DNA sequencing using paraffin blocks. However, some patients diagnosed in advanced stages have only cytology specimens for evaluation. High resolution melting amplicon analysis (HRMAA) is a screening technique which detects missense mutations, deletions and insertions in tumor DNA. In this study, we used HRMAA to identify EGFR mutations in patients with non-small cell lung carcinoma (NSCLC) presenting with positive cytology.

Design: Eleven patients with advanced NSCLC diagnosed by fine needle aspiration were identified. Aspirate smears were reviewed for diagnostic tumor cells and marked with a diamond pen. Marked regions were covered with buffer (1% Tween 20 in 50 mM Tris, 1 mM EDTA, pH 8.0) and scraped with a sterile scalpel. These "slide scrape lysates" were pipetted into a microfuge tube and incubated with Proteinase K for 12-16 hours at 56°C. DNA extracts were amplified using exon specific primers by LightCycler PCR. Samples were then evaluated by HRMAA for mutations in exons 18, 19, 20, and 21. Melt profiles that deviated from wild type were sequenced for confirmation of mutations.

Results: All 11 samples yielded adequate amounts of DNA (ranging from 78-230 ng/μL by spectrophotometer), and all were amplified by PCR using exon specific primers for EGFR. Three cases showed a mutation in exon 19 (del747-753insS); one of these three had an additional mutation in exon 19 (A755D). Nine of 11 cases had a polymorphism for a silent Q787Q mutation in exon 20 (CAG → CAA); of the nine, four were homozygous and five were heterozygous. One case was also heterozygous for a polymorphic silent mutation in exon 21 (R836R, CGC → CGT).

Conclusions: Archival cytology smears from patients with NSCLC can be used to evaluate EGFR mutation status by PCR and HRMAA. The availability of archival cytology smears for mutation analysis increases the potential for clinical management based on minimally invasive biopsy techniques, including possible selection for treatment and review of response to TKI therapy.

281 Prostate Stem Cell Antigen as an Adjunctive Marker for Transitional Cell Carcinoma in Urine Cytology Specimens

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Background: Cytologic evaluation of transitional cell carcinoma (TCC) is notoriously inaccurate, particularly for the diagnosis of low grade lesions. Previous studies utilizing prostate stem cell antigen (PSCA) immunohistochemistry suggested that PSCA increased the sensitivity of detecting TCC in urine cytology specimens. The study, however, was limited by the small sample size, hampering the ability to reach statistical significance. In the current study, a larger retrospective analysis of PSCA expression in 91 archived urine cytology specimens confirms that PSCA immunohistochemistry increases the sensitivity of detecting TCC, especially in low-grade lesions.

Design: The study population included archived urine cytology slides from 45 patients with biopsy proven urothelial carcinoma. Inclusion criteria included urine cytology within 2 months of tissue diagnosis, adequate cellularity, and disease limited to the lower tract. The control population included archived urine cytology slides from 46 patients without a history of TCC. Of the control population, individuals with a history of prostate cancer and inadequate cellularity were excluded. Immunohistochemistry was performed using previously established protocol and scored on a scale of 0 to 3+. Interpreting the urine cytology specimen as "suspicious for urothelial carcinoma" required at least 1+ staining in greater than 25% of cells.

Results: The sensitivity and specificity were 44% and 100% respectively for urine cytology alone. The sensitivity and specificity for detecting TCC with PSCA were 84% and 87% respectively. PSCA alone increased the sensitivity of detecting TCC by 40% ($P<0.001$). However, the specificity decreased by 13% ($P=0.013$) with PSCA. For detecting high grade lesions, cytology had a sensitivity of 71% and PSCA had a slightly better sensitivity of 88%. PSCA staining increased the sensitivity of detecting low grade TCC by 50% when compared to cytology alone ($P<0.0001$) (sensitivity for cytology was 28% versus 78% with PSCA).

Conclusions: PSCA is a promising, noninvasive urine based marker for TCC with increased sensitivity and competitive specificity when compared to cytology alone. PSCA improved the sensitivity for all stages and grades of TCC and with a slightly decreased specificity. As an adjunct to cytology, PSCA markedly improved the detection of low-grade lesions.

282 Imaging Guided Fine Needle Aspiration Biopsy of Solid Renal Masses: A Study of 75 Cases with Follow-Up Histology

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Background: Fine needle aspiration (FNA) of solid renal masses is of particular clinical value when radiology is indeterminate, partial nephrectomy is preferable to radical nephrectomy, or when patients have unresectable neoplasms, such as those with metastases. The role of imaging guided percutaneous FNA has increased, due in large part to the increased detection of small renal masses and the safty and low cost of FNA. This study examines the cytologic-histological correlation, diagnostic accuracy, and potential pitfalls associated with FNA of solid renal masses.

Design: FNA biopsies guided by percutaneous computed tomography of solid renal masses accessioned over the 10-year period of 1996-2005 were reviewed. Only those cases with follow-up histology were included. Diagnostic sensitivity of FNA and concordance between FNA and histology were also evaluated.

Results: A total of 75 specimens with histological follow-up were identified from 75 patients. The cytologic findings were: 66.7% (50 cases) positive for malignancy, 12.0% (9 cases) suspicious for malignancy, 6.7% (5 cases) atypical/possible oncocytic neoplasm, and 14.6% (11 cases) inadequate. The sensitivity of FNA cytology for malignant renal neoplasm was 72.4% (50/69). FNA cytology correctly diagnosed renal cell carcinoma (RCC) in 49 cases and accurately subclassified 93% (27/29) clear cell RCC, 67% (2/3) papillary RCC, 100% (2/2) malignant lymphomas, and 100% (6/6) of metastatic tumors. Subtyping of 10 cases of RCC by FNA cytology was not possible. Eighty-nine % (8/9) of suspicious for malignancy cases had RCC on histology. The 5 cases of atypical/possible oncocytic neoplasm revealed 3 cases of oncocytoma and 2 cases of RCC on histology. Two cases of angiomyolipoma were misdiagnosed on cytology as "favor renal cell carcinoma" and "atypical suspicious for RCC" respectively. Cell block materials for these cases were not available. One case of renal papillary hyperplasia was interpretated as "atypical suggestive of papillary RCC" on cytology.

Conclusions: FNA cytology showed good sensitivity for the identification of malignant renal neoplasms and can accurately classify the tumors by subtype, especially for clear cell RCC, lymphoma, and metastatic tumors. However, renal oncocytic neoplasms and angiomyolipomas are the diagnostically problematic areas, particularly when the cell block material is insufficient for ancillary studies.

283 Urothelial Carcinoma with Associated Proliferative Cystitis: Analyses of ThinPrep Urine Cytology and Fluorescence *In Situ* Hybridization

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Background: Proliferative cystitis (PC), including cytsitis cystica and cystitis glandularis, is a benign reactive urothelial process, and is generally considered to be a diagnostic pitfall in urinary cytology.

Design: All histologically-proven cases of PC with and without urothelial carcinoma (UCA) with urine cytology specimens (UCS) and with fluorescence *in situ* hybridization [FISH (UroVysion, Vysis Inc., Dover's Grove, IL)] were reviewed (2000-6). Papanicolaou-stained ThinPrep (TP) were used for UCS. Appropriate controls were used for FISH.

Results: Clinicopathological material from 16/5700 (0.26%) UCS from 16 patients with PC+FISH (8) and PC+UCA+FISH (8) were reviewed. Eight UCA cases included 5 high-grade UCA (all invasive) and 3 low-grade UCA. Please see Table 1 for data on correlation of UCS and FISH.

Table 1

	Positive urinary cytology	Positive FISH
PC	2/8 (25%)	3/8 (37.5%)
PC+UC	5/8 (62.5%)	4/8 (50%)

"Atypical" included as positive in urinary cytology

Upon review of TP slides, UCS with PC were moderately cellular and demonstrated three-dimensional clusters of benign-appearing urothelial cells with slightly enlarged pale nuclei and small nucleoli. There were two false-positive cases (both diagnosed as atypical). Both cases were considered cytologic overcalls upon review. PC+UCA (high-grade) were hypercellular with clusters and single malignant cells. Nuclei were hyperchromatic and irregular. Nucleus to cytoplasmic ratio was high. There were three false-negative cases, all low-grade UCA. Upon review no malignant cells were seen in these UCS. Overall sensitivity and specificity of TP UCS was 62.5% and 75% and for was FISH 50% and 62.5% respectively.

Conclusions: PC does not pose a diagnostic pitfall in urinary cytology, 75% of PC cases were accurately identified. Role of FISH in cases of PC (with and without UCA) needs further study.

284 Utility of PAX-5 in SLL Cytology: Highly Significant Correlations between Immunomorphology and Flow Cytometry

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Background: Small lymphocytic lymphomas (SLLs) are comprised of monoclonal B cells that characteristically coexpress CD5. Although immunophenotypically aberrant, SLL cells are mature-appearing, posing a challenge to classification by cytology alone. Immunophenotyping (IP) is typically performed by flow cytometry (FC). In some instances, FC is not possible. Immunocytochemical (ICC) interpretation of markers such as CD20 can be difficult in smears due to cytoplasmic fragility. We investigate the utility of PAX-5, the nuclear factor BSAP (B cell lineage specific activator protein), as an ICC adjunct in the diagnosis of SLL.

Design: Archived (n=73) cytologic preparations were retrieved from the ENH files. One slide was selected from each of 42 SLL cases (38 lymph node fine needle aspirations [FNAs] and 4 touch preparations [TPs] from 38 patients, mean 69 yrs, 54% M). Similarly, one slide was selected from each of 31 reactive lymph node (RLN) cases (2 FNAs and 29 TPs from 21 patients, mean 39 yrs, 42% M). FC results were available in 31 (74%) SLL and in 26 (84%) RLN cases. SLL cases lacking contemporaneous IP had prior FC diagnostic of CLL/SLL. All slides were destained and used for PAX-5 (BC/24, Biocare Medical) ICC with antigen retrieval. 500 cells (combined minimum) per slide were assessed by two pathologists (MDC/CDS or MDC/KJK). Cells that were immunomorphologically consistent with SLL were enumerated as PAX-5 positive or negative. Results were compared to historical FC data. An SLL lymph node tissue microarray (n=29 patients) was created (3 cores [0.6 mm] each) and was studied with PAX-5 by IHC as a gold standard.

Results: A remarkably strong direct correlation was found between the % of PAX-5 immunoreactive nuclei in cytology and the % of CD19+ positivity by FC ($R^2=0.841$, $P<0.001$). None of the SLL nodes had less than 60% PAX-5 positive cells on FNA and only one RLN had a PAX-5+ % greater than 59% (Chi Square $p<0.001$). Virtually all SLL tissues in the microarray showed ≥ 90% PAX-5 reactivity by IHC.

Conclusions: In diagnosing SLL by cytology, a strong statistically significant correlation exists between PAX-5 ICC and traditional confirmation by FC. PAX-5 ICC appears to be as useful as IP by FC in distinguishing RLNs from CLL in cytologic samples. Assessing PAX-5 expression by ICC may be clinically relevant in suggesting a diagnosis of SLL in cytomorphologically challenging cases in which IP by FC is not feasible.

285 Two Color Immunocytochemistry for Evaluation of Serous Cavity Fluids

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Background: Evaluation of immunostains in serous fluids by conventional one color immunocytochemistry is challenging because of the difficulty in construction of coordinate immunoreactivity in cell block sections on different slides. Two color immunocytochemistry may simplify interpretation of immunostains of different fluid components in the same section.

Design: We studied 37 serous fluid cytology specimens [23 pleural, 13 peritoneal, 1 pericardial] interpreted as positive for malignant cells with the help of one color immunocytochemistry on cell block sections over a period of 4 years. 3 μm serial sections of cell blocks were immunostained by a dual chromogen method (first peroxidase with brown chromogen, second alkaline phosphatase with red chromogen). Pretreatment and dilution were similar to those used for conventional one color immunostaining. Combinations evaluated were- A: vimentin followed by cytokeratin (CK) 7; B: calretinin followed by BerEP4, C: calretinin followed by CK20. Difficulty of interpretation on a scale of 1 to 5 (1, easy to 5, difficult) was also evaluated.

Results: Combination A showed correlation of the immunoreactivity pattern observed with one color immunostaining (Table 1). The original immunoreactivity pattern of the second immunomarker was compromised in B and C. BerEP4 showed decreased intensity with increased negativity. Immunoreactivity for CK20 was completely lost. The reason(s) for such interference are not clear.

Table 1

Primary neoplasm	Total	CK7/vimentin	%*	BerEP4/calretinin	%*
Breast	13	13	100	7	54
Gastrointestinal	7	7	100	5	71
Pancreas	1	1	100	1	100
Lung	8	8	100	8	100
Ovary	4	4	100	3	75
Peritoneal	1	1	100	0	0
Gynecologic	3	3	100	2	67

* cases in which immunostain combination was diagnostic

Average difficulty of interpretation was 1 with the two color method and 2.95 (range 1 to 5) with the one color method, with a statistically significant difference (two-tailed p value <.0001, paired *t* test). The higher scores of difficulty were observed in cases with a paucity of tumor cells or predominantly single tumor cells.

Conclusions: Dual staining identified the foreign population of malignant cells (Table 1) with significant ease. Immunoreactivity for BerEP4 was diminished and for CK20 was lost. Dual staining increased the ease of interpretation, but proper selection and evaluation of optimum combinations is indicated.

286 Endoscopic Ultrasonography-Guided Fine-Needle Aspiration (EUS-FNA) Combined with Ancillary Techniques in the Diagnosis of Lymphoproliferative Diseases (LD)

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Background: EUS-FNA is an accurate procedure to study gastrointestinal malignancies and stage esophageal and pulmonary neoplasms. The role of this diagnostic approach is not well-known in LD.

Design: To assess the value of EUS-FNA in the diagnosis of LD we studied a series of 15 cases with lymphadenopathy or abdominal masses on imaging studies firstly classified as probable lymphoma based on the on-site cytological exam of the slides. EUS-FNA used a linear echoendoscope and a 22G needle. Part of the samples were preserved for flow cytometry (FC) and molecular studies (M).

Results: See table.

Conclusions: EUS refines other image-based techniques. EUS-FNA may be a useful diagnostic approach for lymphoproliferative disease, but should not preclude surgical biopsy.

Summary of the results of EUS-FNA plus ancillary techniques

Sex / Age	Clinical/imaging information	EUS exploration	FNA information (Cytology/FC/M)	Biopsy
M/71	Pancreatic mass	Coeliac trunk mass	CD5+ small B-cells, lambda restriction; MTC negative	Not done†
F/75	Gastric mass	Normal gastric wall+ perigastric LN	CD5+ small B-cells*	CLL
M/78	Pancreatic mass	Pancreatic mass+ abdominal LN	CD5+/CD23+ small B-cells*	CLL
M/63	Periesophagic mass	Periesophagic mass+ mesenteric LN	CD10+ small B-cells, kappa restriction; MBR positive	FL
F/61	Perigastric mass	Perigastric mass	Small cell population. No abnormal phenotype; policlonal light chains	Castleman disease
F/65	Pancreatic mass	Abdominal LN	CD10+ mixed small-large cells*	DLBCL
M/65	Abdominal LN	Abdominal LN	CD10-, mixed small-large B-cells, lambda restriction;	FL
M/76	Pancreatic mass	Abdominal LN	CD10+, mixed small-large cells*	No diagnostic♦
M/57	Abdominal LN	Abdominal LN	CD10+, mixed small-large B-cells*	FL
F/76	Pancreatic mass	Abdominal LN	Atypical large cells**	Not done♣
M/70	Abdominal LN	Abdominal LN	Large B-cells, no abnormal phenotype	DLBCL
M/34	Abdominal LN	Abdominal LN	Large B-cells; FISH t(8;14) negative	DLBCL
M/54	Retroperitoneal mass	Abdominal LN	Atypical large cells**	DLBCL
M/41	Abdominal LN	Abdominal LN	Atypical large cells**	Atypical mycobacteria
F/76	Mediastinal mass	Mediastinal LN	Reed-Sternberg cells; no abnormal phenotype	Not done

LN, lymphadenopathy; *indicates non-evaluable Ig light chains by FC; **no ancillary studies availabe; MTC, BCL-1 rearrangement; MBR, BCL-2 rearrangement; †treated as aggressive lymphoma, dead of disease ; ♦treated as FL; ♣treated as DLBCL

287 ASCUS Pap Test Results Effectively Correlate with Identification of High-Risk HPV DNA in a High Risk Urban Population: A Clinicopathologic Study of 356 Patients

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Background: Each year approximately 50 million women undergo Papanicolaou testing in the USA. Approximately 3.5 million (7%) are diagnosed with a cytologic abnormality requiring additional follow-up. Liquid-based technology has made reflex human papilloma virus (HPV) DNA testing possible by allowing abnormal specimens to be immediately tested by hybrid capture methodology. The pupose of the present study is to determine whether ASCUS plus HPV DNA testing is useful in a high risk population where the diagnosis of LSIL or HSIL cannot be made on routine screening.

Design: Identification of high risk HPV DNA in 646 consecutive patients with ASCUS was performed by DNA hybrid capture at the Kings County Hospital Center, Brooklyn, NY. Blinded re-review of the orignal cytology was also performed to confirm the diagnosis of ASCUS. Correlation of the cytological diagnosis with the follow-up colposcopy findings and cervical biopsy was also performed.

Results: High risk HPV DNA was detected in 356/646 (55%) of patients with ASCUS. Blinded review of ASCUS cytology in all patients upheld the diagnosis of ASCUS, did not show any HSIL, and an insignificant number of LSIL. Results of colposcopy with biopsy are summarized in Table 1. In some cases, repeat cytology was performed and results are summarized in Table 2.

Conclusions: Based on the findings in this study, we conclude that in high risk patients with a cytologic diagnosis of ASCUS, HPV DNA testing can be useful where the diagnosis of LSIL or HSIL cannot be made on routine screening.

Table 1. Colposcopy and Biopsy Follow-up in High-Risk HPV Positive Patients

Age Group	# of positive HPV	BX follow-up	LSIL	HSIL	Total HPV confirmed by Biopsy	Negative Bx	Other
<25	197	34	21	5	26	8	0
25-34	97	28	12	2	14	13	1
35-44	28	17	7	6	13	4	0
45-54	15	7	0	3	3	3	3
55-64	11	5	2	0	2	2	1
Over 65	8	4	0	0	0	4	0
	356	95	42	16	58	34	3

Table 2. Follow-up Cytology of High-Risk HPV Positive Patients

Age Group	# Positive HPV	Repeated Pap	ASCUS	LSIL	HSIL	Total Persistent HPV	Other
<25	197	16	1	6	1	8	8
25-34	97	4	1	1	0	2	2
35-44	28	2	1	0	0	1	1
45-54	15	0	0	0	0	0	0
55-64	11	2	0	0	0	0	2
>65	8	2	0	0	0	0	2
Total	356	26	3	7	1	11	15

288 Chromosomal Abnormalities Detected by Automated FISH Analysis May Expand the Cytologic Criteria for Urothelial Atypia

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Background: Fluorescence in situ hybridization (FISH) analysis has been shown to be a valuable tool for the detection of urothelial carcinoma. In a routine clinical practice we evaluated the value of reflex FISH testing in the context of classical atypical urine cytomorphology.

Design: Urine samples from 20 patients with atypical cytology were analyzed prospectively with the UroVysion multicolor probe set to chromosome 3, 7, 17, and 9p21. The specimens were stained with Papanicolaou (Pap) and were reviewed by a trained cytotechnologist and a cytopathologist. To support a combined analysis integrating cytology and FISH we used the automated Duet scanning system (BioView) to capture images of all cellular material on these slides. The slides were then decoverslipped, destained and hybridized with the UroVysion probe set. To avoid cytomorphologic bias, a randomized scan was used to classify 250 nuclei. In combined analysis a positive FISH result was defined as ≥5 urothelial cells with a gain of ≥2 of chromosomes 3, 7 or 17 (polysomy, excluding tetrasomy), ≥12 cells with 0 or 1 copy of 9p21, or ≥10 cells with isolated gain of 1 of chromosomes (Chr) 3, 7 and 17. If a case did not meet the positive FISH criteria ≥100 urothelial cells with or without atypia were selected from the captured images and scored for their FISH pattern.

Results: Of 20 patients, 5 (25%) had a history of urothelial carcinoma and only 1 (5%) of these was FISH positive. A total of 8 (40%) patients were FISH positive. Five (25%) patients showed polysomy, 1 (5%) patient showed polysomy and gain of Chr 7, 1 (5%) patient showed gain of Chr 3 and 1 (5%) patient showed loss of locus 9p21. Cells with classic cytologic atypia showed FISH positive results in 6 (30%) cases. Interestingly in 3 (15%) cases chromosomal abnormalities were primarily found in cells without classic cytologic atypia. Negative and positive control cases included in the study for comparison yielded expected results: 2 cytology negative with concurrent FISH negative and 2 cytology positive with concurrent FISH positive.

Conclusions: Reflex FISH testing based on classical cytologic atypia can serve as an important tool in the detection of urothelial carcinomas. Our preliminary results also raise the possibility that chromosomal abnormalities may define a new class of cytologic atypia criteria for FISH testing. Follow up data is required to confirm that this class of cells originates from a clinically undetectable tumor.

289 Role of Age Stratification for Colposcopy Referral Following Initial CIN1 Diagnosis

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Background: Introduction: Referral to colposcopy following a single mildly dysplasia (CIN1) smear is becoming more widely recommended in the developed world. This has workload and cost implications. Aim: To investigate if stratification of CIN1 smears by age group might allow selection of populations that could be followed by repeat cytology initially.

Design: The study set was of all women with a diagnosis of dysplasia between July 2004 and June 2005 in an opportunistic screening programme. The dysplasia was divided into high grade (CIN II and CIN III) and low grade (CIN I) and ratios of high to low grade were calculated for age groups. The age intervals were under 20 years, every five years from 20-34 and every ten years from 35 to 54.

Results: In the study period 34,178 cervical smears were examined. Of these, 2360(6.9%) women were diagnosed with dysplasia, {1580 (4.6%) low-grade, 780 (2.28%) high-grade}. This gave an overall ratio of high-grade to low-grade of 1:2. The ratios stayed relatively constant throughout the age intervals from 20 – 54 years of age, Pearson correlation co-efficient 0.87, p = 0.026.

Conclusions: The ratios of incidences of grades of dysplasia remained constant throughout the age intervals suggesting that selective patterns of referral to colposcopy based on patient's age at diagnosis of CIN1 are not applicable.

Age range	<20	20-24	25-29	30-34	35-44	45-54
Ratio HG:LG	1:4.4	1:2.68	1:1.82	1:1.32	1:1.73	1:2.02

p=0.026

290 Interpretation of Squash Preparations for Central Nervous System Tumors by Cytopathologists: Ability To Distinguish Primary *Versus* Metastasis

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Background: Squash preparations (SqPrep) are commonly used in conjunction with frozen sections (FS) by the neuropathologist (NP) in intraoperative (IO) consultation of central nervous system (CNS) tumors. At our center the SqPrep are stained by Diff Quik (DQ) and H&E stains, analogous to the direct smears used in cytology for on-site adequacy assessment on fine needle aspiration. The aim of this study was to determine if the cytopathologist (CP) is able to accurately interpret CNS SqPrep.

Design: High grade primary CNS tumors or metastases evaluated by SqPrep, FS and permanent histology were selected by the NP for retrospective review. The SqPreps were either stained by DQ, H&E or both. The SqPrep were blindly reviewed by a CP (RSH) utilizing features of background, architecture, nuclei and cytoplasm. The original IO and final diagnoses rendered by a NP (CTW) were compared to the diagnoses of the CP.

Results: Nineteen high grade primary astrocytic tumors (HGPAT) and 19 metastatic tumors with SqPrep were stained with: only DQ, 7; only H&E, 26; or both, 5. The CP diagnosis correlated with the final diagnosis in 35 of 38 cases (92%). The CP correctly identified 18/19 (95%) HGPAT and 17/19 (90%) metastatic tumors. Features suggesting a HGPAT included background: diffuse finely fibrillar or vacuolated lipid material, transgressing vessels (17/19), apoptosis/necrosis (14/19); architecture: *"*feathering*"* of cells along fibrotic bands (2/19); nuclei: enlarged and irregular with fine chromatin (19/19) and small nucleoli (6/19); and cytoplasm: indistinct/processes (19/19). The features of metastatic tumors included background: two distinct cell populations (16/19); architecture: 3-D clusters (13/19); nuclei: round, oval, irregular, hyperchromatic (11/19) and prominent nucleoli (14/19); and cytoplasm: well defined borders/vacuoles (14/19).

Conclusions: The CP accurately diagnosed 95% of primary and 90% of metastatic tumors utilizing distinct morphologic criteria. The features that suggest a HGPAT include a diffuse finely fibrillar or vacuolated lipid background, transgressing vessels, prominent apoptosis, a *"*feathered*"* arrangement of cells, and irregular nuclei with indistinct cytoplasm/processes. The features which best define metastases include two distinct cell populations, 3-D clusters, coarse chromatin, prominent nucleoli and distinct cytoplasmic borders. The ability of the CP to interpret CNS tumors may be valuable in immediate patient management decisions.

291 Analysis of Discordance between Fine Needle Aspirations and Core Biopsies

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Background: Fine needle aspiration (FNA) cytology is a minimally invasive, sensitive and accurate technique for diagnosis of both neoplastic and non-neoplastic lesions. Needle core biopsies (NCB) enhances the sensitivity of percutaneous needle sampling, and there is good correlation between FNA and NCB. The details, however, of the causes of discordance between FNA and NCB have not been reported. This study analyzes 112 cases in which there were discordance between FNA and NCB.

Design: All FNA and NCB were performed by radiologists. At our institution, NCB is not routine but is typically requested by the pathologist only when rapid stain of FNA is non-diagnostic or indicates probable need for ancillary studies such as immunostains. A total of 414 cases that had both image-guided FNA and NCB, performed from January 2003 through July 2006, were retrieved by computer search. Both the NCB and FNA were evaluated and interpreted together, by a cytopathologist. The preliminary interpretation of the FNA, the final findings of both FNA and NCB slides, and probable causes of discordance or failure of FNA to yield a diagnosis were analyzed.

Results: Of 414 cases, 112 had discordance between the final FNA and NCB diagnoses. FNA/NCB sites included: lung (33), liver (19), lymph node (15), mediastinum (9), soft tissue (12), bone (10), kidney (4), and miscellaneous sites (10). One hundred six cases had rapid onsite evaluation using a rapid Romanowski stain. Ninety cases had rapid evaluations interpreted as nondiagnostic. Fourteen were interpreted as positive for carcinoma and 2 as suspicious for lymphoma. The main factors leading to NCB were non-diagnostic rapid preliminary evaluation and need for immunostains. Final FNA diagnoses were as follows: 93 nondiagnostic and 19 malignant. Of the 93 nondiagnostic cases, 24 had a final NCB diagnosis of malignancy, 11 benign tumors, 3 specific infection or granuloma, 53 nondiagnostic, normal parenchyma and/or non-specific inflammation and 2 indeterminate atypical cells. Nineteen malignancies were diagnosed by FNA alone. NCB was nondiagnostic owing to sampling error (15), necrosis (1) or fibrosis alone (1) and no material on the sections (2). Of the 93 nondiagnostic FNA, 48 NCB (51.6%) demonstrated presence of significant fibrosis with or without presence of diagnostic cells.

Conclusions: Our study suggests pathologist-requested NCB is a useful diagnostic stratagem, and that fibrosis is commonly the instigating factor leading to non-diagnostic FNA and diagnostic NCB.

292 Utility of S100P in Diagnosis of Adenocarcinoma of the Pancreas on Fine Needle Aspiration Biopsy Specimens

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Background: Fine needle aspiration biopsy (FNAB) of the pancreas has become a popular approach to establish a definitive diagnosis of pancreatic carcinoma before surgical or other adjunct therapies. Even though the diagnostic criteria for pancreatic ductal adenocarcinoma (PDA) have been well defined, a diagnostic challenge is still present. Attempts have been made to identify specific tumor-associated markers for PDA. However, those markers are usually expressed in normal/reactive pancreatic ductal epithelium as well. Recently, S100P was identified as a specific marker for pancreatic adenocarcinoma, and its utility in FNAB specimens has not been tested and reported.

Design: Using an EnVision-HRP detection kit (Dako), we evaluated the diagnostic value of S100P on 26 cell block sections (CBS) and 36 alcohol-fixed direct smears in 44 cases of FNAB of the pancreas. The 44 cases were divided into 4 groups: Group 1 (G1) – 24 cases (18 CBS and 23 smears) of PDA; Group 2 (G2) – 6 cases (6 smears) with an atypical or suspicious diagnosis; Group (G3) – 9 cases (5 CBS and 5 smears) of benign/reactive pancreatic ductal epithelium; and Group 4 (G4) – 5 cases (3 CBS and 2 smears) of pancreatic endocrine tumor (PET). Clinical followup and/or surgical specimens confirmed the diagnoses. The staining intensity was graded as weak, intermediate, or strong. The distribution was recorded as negative (no staining), 1+ (<25%), 2+ (26-50%), 3+ (51-75%), or 4+ (>75%). Nuclear or nuclear and cytoplasmic staining was regarded as positive. Cytoplasmic staining without nuclear staining was regarded as negative.

Results: Positive immunoreactivity for S100P was observed in 18 of 18 PDA cases in CBS and 23 of 23 de-stained slides, with 3+ and 4+ staining in 17 cases and 22 cases, respectively. All cases in G3 and G4 were negative for S100P, with the exception of 1 case of benign glandular epithelium on the de-stained slide that showed positive staining and strong background staining. Importantly, all atypical/suspicious cases in G2 were positive for S100P with greater than 2+ positivity (clinical followup confirmed carcinoma in all cases).

Conclusions: Our data indicate that S100P is a sensitive and specific marker for detection of PDA on FNAB specimens on both cell block sections and direct smears, but caution should be taken in interpreting positive results on de-stained smears because background staining may be present.

293 Value of Galectin 3 in Diagnosis of Atypical Thyroid Lesions in Fine Needle Aspiration Biopsy Specimens

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Background: Fine needle aspiration biopsy (FNAB) of the thyroid under radiologic guidance has become a popular approach to establish a definitive diagnosis prior to surgical intervention. As a result, many so-called papillary thyroid microcarcinoma (PTMC)(tumor size <1.0 cm) are being sampled. Because of the limited number of papillary carcinoma cells in PTMC specimens as compared to classic papillary thyroid carcinoma (PTC) specimens (tumor size >1.0 cm), many of these FNABs are diagnosed as atypical. The incidence of PTMC has been reported to be about 30% of that of PTC. PTMC has been regarded as clinically insignificant; however, more recent data suggest that PTMC should be managed as classic PTC. Galectin 3 has been shown to be a sensitive and specific marker for PTC. In this study, we investigated the utility of galectin 3 in the detection of the limited numbers of papillary carcinoma cells in FNAB specimens.

Design: We evaluated the diagnostic value of galectin 3 in alcohol-fixed direct smears and/or cell block sections (CBS) from 42 FNAB specimens of the thyroid. The 42 cases were divided into 3 groups: Group 1 (G1) – 12 cases (12 smears, 10 CBS) positive for PTC; Group 2 (G2) – 19 cases (19 smears, 11 CBS) with atypical diagnosis; and Group 3 (G3) – 11 cases (11 smears, 11 CBS) with benign/reactive diagnosis. The staining intensity (weak or strong) and the distribution (negative, 1+, 2+, 3+, or 4+) were recorded.

Results: Follow-up surgical specimens confirmed the presence of papillary carcinoma in all cases in G1, with an average tumor size of 1.8 cm (ranging from 0.7 – 4.0 cm, with only one tumor less than 1.0 cm). Positive reactivity for galectin 3 was observed in all 12 cases in G1, in both smears and CBS, with 3+ or 4+ staining in 10 of the 12 cases. Eleven cases in G2 were surgically confirmed as positive for papillary carcinoma, with a tumor size of less than 1.0 cm (ranging from 0.1 – 0.9 cm, mean 0.65 cm) in 8 cases. Positive immunoreactivity for galectin 3 was also observed 9 of these 11 positive specimens, with 3+ or 4+ staining in 5 of the 9 cases. Of the 8 negative cases in G2, 4 were Hashimoto's thyroiditis (HT) and 4 showed follicular adenoma. Two of the 8 cases were focally positive (1-2+), and both showed HT. All cases in G3 were negative for galectin 3.

Conclusions: The results indicate that galectin 3 is a relatively sensitive and specific marker for the detection of PTMC in FNAB specimens in both CBS and smears. However, caution should be taken because HT may also be focally positive for galectin 3.

294 Cytohistologic Correlation of ASC-H Diagnosis and HPV in Adolescents and Young Adults

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Background: Atypical squamous cells - cannot exclude high-grade squamous intraepithelial lesion (ASC-H) has been linked to high-grade cervical intraepithelial neoplasia. We have recently demonstrated an increase in the frequency of ASC-H diagnosis in adolescents and young adults, which parallels the higher frequency of

positive test for high risk human papilloma virus (HR-HPV) in this group. However, the cytohistologic correlation of ASC-H diagnosis in adolescents and young adults and the frequency of high risk HPV in this group have not been investigated.
Design: In our current study we evaluated the histological follow-up in 100 adolescents and young adults (age range: 12-21 years) diagnosed with ASC-H and evaluated the presence of HR-HPV.
Results: A total of 592 women with ASC-H Pap tests were diagnosed over a 10 months period;17 % (100/592) of which were adolescent and young adults. Follow up biopsy or curettage was performed in 59 of the 100 patients, and revealed reactive/negative findings in 10 of 59 (17%) patients; CIN 1 in 44/59 (75%) and CIN2 in 5/59 (8%). No CIN 3 lesions were detected. Test for HR- HPV was performed in 49 of the 100 ASC-H Pap tests. 32 of 49 (65%) were positive HR-HPV, 15/49 (30%) were negative and 2/49 (4%) samples were insufficient for HPV evaluation.
Conclusions: Our data demonstrated that 8 % of adolescents and young adults with ASC-H diagnosis will harbor a significant lesion (CIN2); these pathologic findings may require medical management or close clinical observation as these lesions in adult patients have been reported to regress.

295 A Simplified Method for Correcting Verification Bias Encountered during Assessing Diagnostic Performance of a Cytologic Test

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Background: In assessing the diagnostic performance of a cytologic test during routine use, the final disease status of study subjects is often unknown and verification of the disease is biased by the result of the test itself. For example, subjects with metastatic disease and a positive FNA may not have a tissue diagnosis that verifies the FNA result. Similarly, a negative HPV test passes unconfirmed. Few complicated techniques have been suggested to correct for this bias. In this study, we used a simple Excel-based calculation to correct for verification bias encountered during assessing the performance of cytologic tests.
Design: We selected two cytologic tests: EUS-FNA and HPV test. All consecutive EUS-FNA from 04/00 to 04/04 and which were followed up till 12/04 were included in the study. Also included all HPV tests submitted from 01/00 till 09/05 and followed-up till 08/06. With histologic diagnosis cosidered as the "gold standard", unocrrected sensitivity and specificity with confidence intervals (CI) were calculated. To correct for verification bias, the data was presented as 2X3 with additional raw indicating unverified cases. The formulas for corrected sensitivity, specificity and CI interval according to Begg and Greenes were entered in a spread sheet. The latter were then compared and considered difference when there is no overlapping.
Results: The uncorrected sensitivity and specificity (CI) for EUS-FNA are 93% (87-97%) and 97% (70-86%), reprectively, while the corrected ones are 95% (92-99%) and 74% (66-82%), respectively, Table 1. The uncorrected sensitivity and specificity (CI) for reflex HPV test are 91% (84-95%) and 24% (21-28%), respectively, while the corrected ones are 66% (64-68%) and 63% (47-78%), respectively, table 2. The bias corrected sensitivity and specificity were not significantly different than uncorrected sensitivity and specificity for EUS-FNA but not for HPV reflex HPV.
Conclusions: Depending on the size of unverified diagnoses, verification bias significantly impact performance indices. The magnitude and significance of this bias can be determine using a simple spreadsheet calculation.

EUS-FNA

		Test +	Test -	Total
Verified	Disease +	98	7	105
	Disease -	21	80	101
Unverified		266	122	388
Total		385	209	594

Reflex HPV

		Test +	Test -	Total
Verified	Disease +	90	9	99
	Disease -	516	166	682
Unverified		1335	3186	4521
Total		1941	3361	5302

296 Multi-Test Reevaluation of Negative HPV Results Following Abnormal Cervical Cytology: Do HPV Negative Samples Exist?

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Background: Human papillomavirus (HPV) is accepted as a necessary cause of cervical carcinoma. Virtually all cervical cancers have tested HPV positive after examination with multiple alternative assays. The prevalence of HPV in cervical cytology samples has not been subject to a similar multi-test approach.
Design: In a previous study, 200 ASC-US, 200 LSIL, 200 ASC-H, 199 HSIL, and 300 NILM samples were tested for HPV using a modification of the GP5+/6+ PCR assay. The aim of this study was to test the 'GP5+/6+ negative' abnormal cervical cytology samples and an age-matched cohort of similarly negative NILM samples with three or four additional PCR assays. All samples were assessed using the FAP59/64, LCR-E7, and PGMY09/11 systems. Any samples negative after PGMY09/11 PCR were subject to PGMY09/11-GP5+/6+ nested PCR. HPV type was determined by cycle sequencing of PCR products.
Results: DNA samples were available for 45/47 abnormal samples (20 ASC-US, 5 LSIL, 14 ASC-H, 6 HSIL) from the previous study. On further testing, HPV was demonstrated by one or more of the FAP59/64, LCR-E7, or PGMY09/11 assays in 41/45 (91%) abnormal cytology samples, and in 10/47 (21%) NILM samples (P<0.0001). HPV types 20, 32, 39, 51, 52, 53, 54, 56, 62, 68, 73, and 82 were detected among the abnormal samples; HPV types 30, 42, 53, 54, 62, 74, 87, 90, and FA79 were detected among the NILM samples. Following nested PCR an additional two abnormal cytology samples tested positive (both HPV66), and an additional twenty-five NILM samples tested HPV positive for types 16 (two different variants), 18, 31, 54, 66, 81 or 84. Thus, following all four assays, 43/45 (96%) abnormal samples tested HPV positive and 35/47 (74%) NILM samples tested HPV positive (P<0.01). The data also showed different HPV type detection propensities among the PCR assays.
Conclusions: Together with our earlier study, the data demonstrate HPV in 795/797 (99.8%) abnormal cervical samples consistent with HPV representing a necessary cause of abnormal cervical cytological conditions. The high percentage of NILM samples that tested positive only after application of a highly sensitive nested PCR approach suggests the possibility of the detection of latent HPV. The strengths and limitations of HPV PCR assays should be well characterized before their implementation as clinical tests.

297 Comparison between Conventional and Thin-Layer Cytology in Thyroid Lesions: Institutional Experience with 10,360 Cases

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Background: Fine needle aspiration biopsy (FNAB) represents the most reliable diagnostic tool in the diagnosis of thyroid lesions. The efficacy of thyroid cytology on FNAB processed by thin layer cytology (TLC) compared to conventional smears (CS) is evaluated in 3 reference periods based on the use of CS alone, both CS and TLC, and TLC alone.
Design: The efficacy of TLC alone compared to both TLC and CS in the same case and the CS alone in 10,360 thyroid lesions was evaluated in 3 reference periods. In the biennium 2004-2005, 4,522 FNABs were processed only with TLC, 3,442 had both CS and TLC in the period 2001-2002 and 2,396 only with CS in the period 1997-1998. These parameters of efficacy were chosen: rate of inadequacy (IR), rate of indeterminacy (INR) corresponding to the diagnosis of follicular neoplasm/lesion and rate of malignancy (MR).
Results: IR was 8.8% in 1997-1998, 18% in 2001-2002 and 13.1% in 2004-2005.MR was 2% in 1997-1998, 2.5% in 2001-2002 and 2.1% in 2004-2005. INR was 14.4% in 1997-1998, 21.4% in 2001-2002 and 14.5% in 2004-2005. The differences in IR in the earliest biennium compared to the following resides mainly in the fast check of the adequacy of CS slides done by the cytopathologists. The use of TLC and the increase of FNABs caused the discontinuation of adequacy control. The similarity of MR in all periods is an evidence of the diagnostic efficacy of TLC and CS.The trend of INR could be explained by the change of method (from CS to TLC) which reduces, at least in the training period, the self-confidence of the pathologist.
Conclusions: TLC alone decreases the number of both inadequate and indeterminate diagnoses without modifying the MR.The wide application of immunocytochemistry (ICC) on TLC may reduce the INR. The impossibility of the adequacy control during the FNAB suggests two passes for each lesion and the preparation of a second TLC slide in case of scant cell groups.

298 Touch Imprint Cytology of Core Needle Biopsy of Thoracic, Abdominal, and Head and Neck Masses: A Review of 87 Cases

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Background: Increasing use of CT guided core needle biopsies in recent years has resulted in on site evaluation of core biopsy with touch imprints to ensure adequate sampling and render preliminary diagnosis. We review our experience of core needle biopsy specimens from thoracic, abdominal and head and neck masses where concurrent touch imprints were made.
Design: Core needle biopsies (CNB) performed under CT guidance with concurrent touch imprint cytology (TIC) on all patients over a period of 5 ½ years (January 2001 to June 2006) were reviewed. The aim of the study was to assess the accuracy of TIC predicting benign or malignant histology on CNB.
Results: Of the 87 CNB analyzed 58 (67%) were malignant (39 thoracic, 14 abdominal, and 5 head and neck) and 29 (33%) were benign (16 thoracic, 10 abdominal, and 3 head and neck). The patients ranged in age from 3 to 86 years (mean 60). Touch imprint cytology was categorized as malignant 52 (60%), atypical 4 (5%), suspicious for malignancy 2 (2%) and benign 29 (33%). Malignant, atypical and suspicious for malignancy on TIC were combined as one category for statistical analysis. Touch imprint cytology accurately predicted the final benign or malignant histology in 83 (95%) cases, with a sensitivity of 96% and a specificity of 93%. One false positive resulted from the interpretation of benign chondrocytes as atypical cells. The other false positive resulted from numerous plasma cells in an area of chronic inflammation, being interpreted as a possible plasma cell dyscrasia. The two false negatives were likely the result of inadequate TIC.
Conclusions: Touch imprint cytology is highly a sensitive and specific procedure for predicting benign or malignant histology on CNB. Immediate assessment on TIC may also facilitate triaging of patients for further management and core material for ancillary testing.

299 Is It Pancreatic or Ovarian Carcinoma in Peritoneal Fluid: A Short and Practical Immunohistochemical Approach

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Background: Metastatic adenocarcinoma is commonly seen in the peritoneal fluid. In most cases, previous history is known and confirmatory immunohistochemical studies are not necessary. But in some occasions, malignant ascites with unknown primary can be challenging. Pancreas and ovary are among the organs that are usually evaluated as a source of primary. The purpose of this study is to investigate a short panel of immunohistochemical staining to help differentiate pancreatic from ovarian carcinoma.

Design: A cohort of 20 cases of peritoneal fluid with evidence of metastatic carcinoma was found where cell block material was available and adequate to perform the intended immunohistochemical studies. The group included 7 patients with confirmed pancreatic carcinoma, 11 with well-documented ovarian carcinoma and 2 had malignant ascites that was considered of unknown primary. The panel of immunohistochemical stains included: MUC1, MUC2, MUC5a, WT-1 and CA-125.

Results: The results are shown in the following table.

Immunohistochemical Staining of Pancreatic and Ovarian Carcinoma in Peritoneal Fluid

	MUC 1	MUC 2	MUC5a	WT-1	CA-125
Pancreatic Ca (n=7)	7/7 (100%)	0/7 (0%)	7/7 (100%)	0/7 (0%)	4/7 (57%)
Ovarian Ca (n=11)	11/11 (100%)	0/11 (0%)	0/11 (0%)	10/11(91%)	7/11 (64%)
Unknown Primary (n=2)	2/2 (100%)	0/2 (0%)	0/2 (0%)	1/2 (50%)	2/2 (100%)

Conclusions: The combination of MUC5a positivity / WT-1 negativity was seen in 100% of pancreatic carcinoma while MUC5a negativity / WT-1 positivity in 91% of ovarian carcinoma. It is believed that this short panel of immunohistochemical stains is useful in distinguishing pancreatic from ovarian carcinoma in body fluid cytology.

300 Anal Pap Smears: Cellularity and Role of HPV Testing

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Background: Anal Pap smears have recently gained popularity as screening tests for HPV associated dysplasia in HIV+ patients. The diagnostic criteria for dysplasia in these samples are not well defined and it's utility for detecting high grade lesions is relatively unproven. We experienced a marked increase in Anal Pap smear volume at our institution beginning in late 2005 and sought to understand it's clinical utility and correlation with HPV testing.

Design: 131 consecutive Anal Pap smears from 38 women and 93 men (86% with a referring diagnosis of HIV immunodeficiency) that were performed between 2004 and 2006 were processed for thin layer cytology using CytoRich collection medium (TriPath Oncology). All samples were subjected to a Hybrid Capture II assay (Digene) for high-risk HPV. Two cytopathologists independently scored samples as negative, indeterminant, or positive for dysplasia. Sample cellularity (average number of cells per high-power field) was also assessed.

Results: 39% of samples were of adequate cellularity using the Bethesda criteria for cervical liquid-based preparations. The consensus cytologic diagnoses were as follows: 22% positive, 24% indeterminant and 54% negative. Overall interobserver agreement was substantial (89%, weighted kappa = 0.74), however, agreement on indeterminant samples was minimal (kappa = 0.02). The frequency of negative, indeterminant, and positive cytologic diagnoses varied significantly with specimen cellularity (chi-square = 42.3, $p < 0.01$) with no positive diagnoses in the lowest cellularity samples. 39 samples (28%) were positive or borderline for high risk HPV and there was significant agreement between HPV and cytologic diagnosis (Chi square =67.3, $p < 0.01$). HPV results did not differ significantly with sample cellularity (Chi square = 11, $p > 0.05$). Five of 80 low cellularity samples (6%) were positive or borderline positive for HPV. Two of 51 adequate cellularity samples (4%) were positive for dysplasia but negative for HPV.

Conclusions: Interobserver agreement for positive and negative cytologic diagnosis and correlation with high risk HPV testing was fairly good for Anal Pap smears but the concordance on indeterminant samples was minimal. Low specimen cellularity compromises the diagnostic accuracy of Anal Pap smears but concurrent HPV testing permits identification of some false negative samples.

301 Estimation of Nuclear Area in Atypical Squamous Cells of ThinPrep Pap Tests

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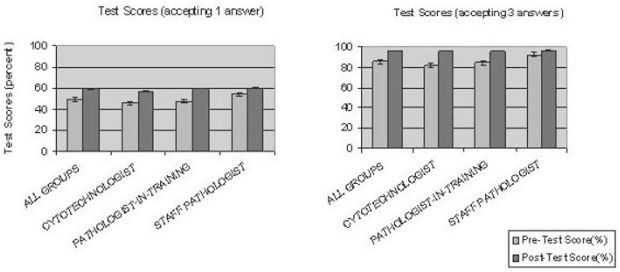
Background: The Bethesda System diagnosis of "atypical squamous cells of undetermined significance" (ASC-US) is dependent on several criteria, including "nuclei are approximately two and one half to three times the area of the nucleus of a normal intermediate squamous cell." Since in clinical practice the criteria are usually employed in aggregate, pathologists seldom consciously calculate this area. This study addresses how well individuals estimate nuclear size ratios and if the ability improves with a short training session.

Design: Volunteers were recruited from staff pathologists, pathologists-in-training, and cytotechnologists. Digital photomicrographs at 400x were taken, and two nuclei in each image were indicated by arrows. An inital exam consisted of twenty-five images and subjects were asked to estimate the area ratio of the two indicated nuclei as one of five categories (<1.50, 1.50-2.00, 2.01-2.50, 2.51-3.00, and >3.00), with responses compared to calculated ratios. An instructional session followed, consisting of fourteen additional photomicrographs which displayed the calculated ratio of the two nuclei. A second exam of twenty-five different photomicrographs followed, including the same number of each of the five categories of nuclear size ratio.

Results: Two scores for each group were calculated: 1) percentage of cases answered identically to calculated ratio, and 2) percentage answered within one category of calculated ratio (i.e., if the calculated ratio category was 2.51-3.00, then 2.01-2.50, 2.51-3.00, and >3.00 were all accepted). The subjects as a whole showed a statistically significant increase after the instructional session; of the individual groups, only the pathologists-in-training group showed a significant increase. Using the broader scoring definition, a significant increase was seen in both pathologists-in-training and cytotechnologists. Staff pathologists had the highest scores both before and after the instruction set.



Conclusions: Evaluators were able to reasonably estimate nuclear areas measured independent of other cytologic features, with improved scores after training. Pathologists-in-training showed the greatest improvement.

302 Interobserver Variability (IOV) in HPV Test Results in Pap Tests Interpreted as Atypical Squamous Cells (ASC)

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Background: Annually, millions of Pap tests are interpreted as ASC of undetermined significance (ASCUS) and as ASC, cannot exclude high grade squamous intraepithelial lesion (SIL) (ASC-H). The results of human papillomavirus (HPV) DNA testing is valuable in triaging women with an ASCUS diagnosis (dx). HPV results may also function as a quality monitor of the frequency of ASC dxs.

Design: We examined the IOV in the proportions (%) of HPV positive (+) results (performed by PCR) for ASCUS and ASC-H dxs among 5 pathologists from the same lab over a 2 year period (2004 and 2005), using Chi square analysis. All Pap tests were liquid based.

Results: 33% of the 1299 ASCUS dxs had HPV testing; 48% were positive (88% were high risk [HR] viruses). The % + varied by pathologist from 35 to 67%, and this difference was significant ($p=0.026$) for all cases and for those with HR viruses. The pathologist with a 35% + rate had an ASCUS/SIL of 0.58; the one with 67% had a ratio of 1.02. 33% of the 222 ASC-H dxs were HPV tested and 63% were + (98%were HR types). No significant differences in ASC-H proportions were found, although the % + ranged from 54 to 83%.

Conclusions: Within the same lab, IOV exists in the % HPV + ASCUS and ASC-H dxs. This may represent unstandardized use of ASC dxs. Benchmarking of this % remains to be determined, as does the relationship of the % HPV + cases and the ASCUS/SIL ratio.

303 Retrospective Review of Thyroid Fine-Needle Aspirations: Does Pathologist's Experience Predict Cytologic-Histologic Correlation?

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Background: Fine-needle aspiration (FNA) of the thyroid remains the most non-invasive, accurate, cost-efficient pre-operative method for distinguishing benign from malignant thyroid nodules. Multiple studies addressing accuracy of thyroid FNA have offered varying reasons for cytologic-histopathologic discordance, the majority of which may be attributed to the known limitations of thyroid FNA. No previous study has formally addressed accuracy of thyroid FNA with respect to the interpreting pathologist's level of expertise.

Design: A retrospective review of 392 consecutive thyroid excisions at a large medical center was performed. Corresponding cytology reports (113 intra-institutional,151 extra-institutional) were retrieved for 264 of the 392 cases. Accuracy of FNA interpretation of the 4 intra-institutional hospital-based pathologists (2 with fellowship training/specialty certification in cytopathology, 2 with practical expertise and dedicated interest in thyroid cytology) was compared with that of multiple extra-institutional pathologists staffing a commercial laboratory.

Results: Cytologic-histologic correlation of the institutional and extra-institutional cases demonstrated concordance rates of 92% and 82%, respectively, and discrepancy rates of 5% and 8%, respectively. Sensitivity, specificity, negative predictive value, and positive predictive values for the institutional and extra-institutional cases were compared (**Table 1**). FNA aspirates were more likely to be interpreted as indeterminate by extra-insitutional pathologists, and outside indeterminate aspirates were more likely to be a non-neoplastic process or benign neoplasm than a malignant neoplasm, on surgical excision.

Conclusions: In this investigation, the aspirates interpreted by pathologists with specialized training and expertise demonstrated greater concordance with higher specificity, higher predictive value, and fewer indeterminate diagnoses. As indeterminate diagnoses generally result in surgical excision, a second opinion by a pathologist experienced in interpreting thyroid FNA should be considered.

Table 1- Cytologic-histologic correlation

	Sensitivity	Specificity	Positive predictive value	Negative predictive value
Overall FNA cases (n=264)	84%	88%	81%	90%
Institutional FNA cases (n=113)	80%	100%	100%	87%
Extra-institutional FNA cases (n=151)	87%	80%	69%	92%
	p=0.1248	p<0.001	p<0.001	p=0.1829

304 Utility of Anti-L523S Antibody in the Diagnosis of Benign and Malignant Pleural Effusion

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Background: The key to the diagnosis of malignancy in pleural effusion is to identify "foreign" cells with atypia. Certain conditions producing benign pleural effusion can cause cytological atypia in mesothelial cells. Distinguishing malignant cells from reactive mesothelial cells in pleural effusion may be difficult. Immunohistochemistry, special stains and electron microscopy are helpful in distinguishing metastatic carcinoma from atypical mesothelial cells, but they are not useful in separating reactive from malignant mesothelial cells. K homolog domain containing protein overexpressed in cancer (KOC), a member of the insulin-like growth factor mRNA-binding protein (IMP) family, also known as L523S and IMP3, is expressed during embryogenesis and in certain malignancies. We analyzed immunostaining results to determine the utility of anti-L523S antibody, clone 69.1 in differentiating reactive mesothelial cells from malignant cells in pleural effusion.

Design: 51 cases with paraffin embedded pleural effusion cell blocks were retrieved, previously diagnosed as benign (16) or malignant pleural effusion (35, 10 malignant mesotheliomas, 10 lung adenocarcinomas, 7 breast adenocarcinomas, 4 colonic adenocarcinomas, 3 small cell carcinomas and 1 squamous cell carcinoma). Immunohistochemistry was performed for KOC, calretinin and CK5/6. Staining intensity for KOC was scored from 1-3 (3 being the strongest), and calretinin and CK5/6 positivity was recorded.

Results: In 35 malignant pleural effusion samples positive staining for KOC in a variable degree of intensity was observed in 28 (80%) cases, including 100% of mesotheliomas, 100% of lung adenocarcinomas, 100% of small cell and squamous cell carcinomas, 75% of colonic adenocarcinomas and 14% of breast adenocarcinomas. 16 cases diagnosed as reactive mesothelial cells showed positivity for calretinin and 13/16 were CK5/6 positive. Interestingly, mesothelial cells were positive for KOC in 2/16 cases originally interpreted as benign. One of these was diagnosed as malignant mesothelioma on pleural biopsy. All malignant mesotheliomas were positive for calretinin, and 8/10 cases had CK5/6 staining.

Conclusions: Anti-L523S antibody is a sensitive and specific marker for detection of malignant cells in pleural effusion, and it has significant utility in diagnosis of reactive mesothelial cells, malignant mesothelioma and metastatic carcinoma in combination with calretinin and CK5/6 staining.

305 Estimated False Negative Results of HC2 HPV Testing as Correlated with Consensus Pap Test Diagnosis

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Background: Hybrid capture II (HCII) test (Digene Corporation) has been approved by FDA for detection of Human Papilloma Virus (HPV) as a screening test for women at risk for cervical neoplasia. The level of virus detection by HCII is rated at 5000 viral copies or 1 picogram of HPV DNA per sample. HPV DNA testing is not only a useful adjunct to the Pap test in cervical cancer screening, but it also has been suggested as a monitor of quality assurance in Pap screening. The sensitivity of HCII has been reported as high as 100% in some clinical trials, however, the test performance and false negative error in routine testing in hospital settings still needs to be evaluated. The purpose of this study was to determine the false negative rate of HPV test by HCII test in bona fide High Grade and Low Grade Squamous Intraepithelial Lesions (HSIL and LSIL) on ThinPrep Pap Tests.

Design: The study group included women aged 24-91 years of age. All HPV negative LSIL and HSIL cases over a 30-month period from 2004 to 2006 were re-screened by a certified cytotechnologist and, independently, by an expert cytopathologist. From the number of actual squamous intraepithelial lesions (SIL) that had negative HPV tests the percentage of false negatives in both LSIL and HSIL were calculated.

Results: A total of 193 HSIL and 1515 LSIL were reflexed for HPV DNA testing. In 192 HSIL and 1513 LSIL cases the diagnoses were confirmed upon re-review**.** In1HSIL and 2 LSIL the diagnosis was downgraded to ASCUS upon re-review and these cases were excluded. HPV HCII test results: HSIL cases – 91.1% positive, 6.3% negative, and 2.6% unsatisfactory for HPV testing; LSIL cases: 86.9% positive, 10.9% negative, and 2.0% unsatisfactory for HPV testing. The false negative rates of HPV HCII testing were 6.25% for HSIL ,10.97% for LSIL and 10.45% for all SIL. The likely reason for the negativity in LSILs is noninclusion of 6 low risk HPV types in the low risk HCII cocktail probe, and a low viral load in HSILs.

Conclusions: HPV DNA testing by HCII is used in many laboratories as a monitor of quality assurance in Pap screening. While a sensitive test, HCII has a false negative error of up to 10% in the routine screening, which has to be taken into account during correlations of Pap tests and HPV tests results.

306 The Role of Pre-Operative Axillary Ultrasound and/or Ultrasound Guided Fine Needle Aspiration in the Management of Breast Cancer Patients

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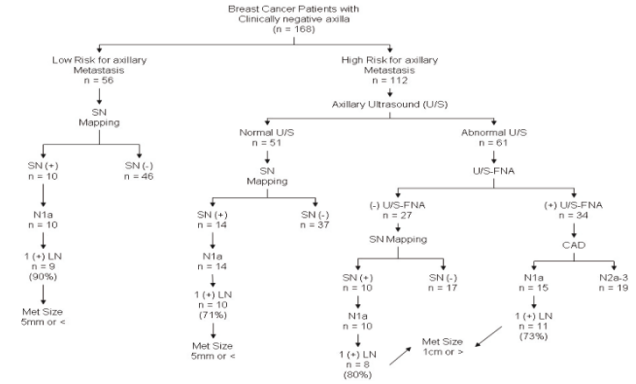
Background: Sonographic (U/S) evaluation of the axilla can predict final node status in a significant percentage of clinically node negative (-) patients. This study assesses the value of pre-operative U/S followed by U/S guided Fine Needle Aspiration (U/S-FNA) in determining conservative versus complete axillary dissections (CAD).

Design: Data on breast primaries from 168 consecutive sentinel node (SN) candidates were prospectively assessed for clinicopathologic variables associated with increased risk of axillary metastases*. Patients in which these variables were identified underwent U/S of their axillae followed by U/S-FNA when abnormal nodes were detected. SN mapping was done in patients with normal axillary U/S or (-) FNA. Patients with positive (+) FNA proceeded to CAD. The axillary status of patients not meeting the high-risk (HR) criteria was also recorded and the number of (+) axillary nodes in the two groups was compared. *HR criteria: Patients with grade (G) III, >1cm and G II, >1.5cm (+/- lymphvascular invasion) breast primaries on histology and patients with high nuclear G, >1.5cm primaries on FNA (dimensions of primary tumors were based on mammographic or U/S size).

Results: 70% (n=44/61) of the HR patients with abnormal axillary U/S had at least 1 (+) node on final histology, 56% (n = 34/61) of which were (+) by FNA. Of the latter 56% (n = 19/34) had >3 (+) nodes (N2-3) with metastatic deposits >1cm. 16% (n = 10/56) of the low risk (LR) patients had (+) axilla, 90% with metastatic deposits < 5 mm. in a single (+) SN (N1a). Likewise, the 21% (24/112) of HR patients with normal U/S and or (-) FNA that had (+) axilla were all N1a, 75% (18/24) with a single (+) SN and metastatic deposits <5mm and >1cm respectively.



Conclusions: Classifying SN patients into HR and LR based on our criteria is clinically valid. LR patients, and patients with (-) axillary U/S and/or (-) FNA may benefit from less aggressive axillary dissections. HR patients with (+) U/S-FNA should undergo CAD.

307 Utilization of ASC-H, LSIL, Cannot Exclude HSIL (LSIL-H) and HSIL Terminology Following Implementation of the ThinPrep Imaging System

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Background: Automated computer imaging systems function to improve cytopathology lab performance in the detection of cervical epithelial abnormalities. The ThinPrep Imaging System (TIS) (Cytyc Corp., Marlborough, MA) algorithms are designed to be more sensitive than a cytotechnologist at detecting small single cells and hyperchromatic groups, at a cost to specificity. The diagnosis of ASC-H relies on detection of these small cells and hyperchromatic crowded groups. Prior to the implementation of TIS, the ASC-H rate was .17%. The purpose of this study is to compare ASC-H, LSIL-H and HSIL after one and one-half years of experience using the TIS in the cytopathology laboratory.

Design: All Pap tests performed at our institution from January 1 - December 31, 2005 were retrieved from the computer database. Pap tests for this study were interpreted by one of eight staff pathologists. Each Pap test interpreted as ASC-H, LSIL-H or HSIL was studied to determine histologic and Pap test follow-up. For each case, the most severe Pap test interpretation or histologic diagnosis was recorded. Available HPV results were also recorded for ASC-H patients. HPV testing was performed using the Hybrid Capture 2 Method (Digene Corp., Gaithersburg, MD).

Results: Of 62,047 Pap tests performed in 2005, 278 were interpreted as ASC-H (.45%) 135 as LSIL-H (.22%) and 226 as HSIL (.36%). Comparative Pap test and histologic follow-up for these interpretation is presented in Table 1. Of 209 ASC-H patients with HPV results, 99 were negative (47%), 5 were equivocal (3%) and 105 were positive (50%). Of 29 LSIL-H patients tested for HPV, 7 (24%) were negative, 1 (4%) was equivocal and 21 (72%) were positive.

Patient Follow-up for ASC-H, LSIL-H and HSIL

	ASC-H # (%)	LSIL-H # (%)	HSIL # (%)
Negative follow-up	98/215 (46)	24/100 (24)	33/190 (17)
CIN1/LSIL	34/215 (16)	32/100 (32)	24/190 (13)
CIN2-3/HSIL +	56/215 (26)	35/100 (35)	128/190 (67)
Atypical PAP follow-up	27/215 (12)	9/100 (9)	5/190 (3)

Conclusions: Use of the TIS has led to increased ASC-H interpretations in our laboratory. Because of this probable increased detection of small cells and groups of cells using the TIS, the overall patient follow-up in these ASC-H cases shows fewer HSIL/CIN2-3 than were identified prior to TIS implementation. Two-thirds of patients with LSIL-H had either CIN1 or CIN2-3+ on follow-up. Likewise, two-thirds of patients with HSIL interpretations had CIN2-3+ on follow-up.

308 Cytopathology of Extraskeletal Myxoid Chondrosarcoma: A Report of 7 Cases

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Background: Extraskeletal myxoid chondrosarcoma (EMC) is an uncommon soft tissue neoplasm. With the exception of a series of 5 cases, the cytopathology of this tumor is largely limited to single case reports. The aim of our study was to evaluate a series of EMC cases obtained by imprint/scrape cytology and fine-needle aspiration (FNA) biopsy.

Design: We reviewed our cytology files for all soft tissue lesions signed out as chondrosarcoma and EMC, and our tissue files for any cases of EMC that had corresponding cytopathology. FNA was performed using standard technique. Scrape preparations were performed from tissue sent fresh to the laboratory for either frozen section, or for special studies such as electron microscopy or tissue banking.
Results: Seven cases of EMC were retrieved from 4 men and 3 women (x age=62 yrs.). All patients had histologic confirmation of the diagnosis of EMC. Only 3 individuals had a prior diagnosis of EMC. Sites included 4 cases from the foot/ankle, 1 wrist, 1 calf, and 1 buttock. Four cases were diagnosed from FNA biopsy, while 3 were diagnosed from scrape slides. Six were correctly and specifically diagnosed as EMC, and 1 was diagnosed as myxoid spindle/epithelial neoplasm. Cytologic features included hypocellular to highly cellular smears of rounded cells set in an abundant myxoid stroma that varied from opaque to semi-transparent, and lacked vascularity or necrosis. Most smears showed cells in short, sometimes anastomosing cords, but also as single cells and cells clusters. Cells displayed a monotonous uniformity in nuclear diameter and cell size. Bland nuclei with evenly dispersed chromatin displayed small distinct nucleoli and a small amount of infrequently vacuolated cytoplasm. Tissue fragments were found in 3 of 4 FNA cell-blocks. FISH analysis using the *EWSR1* probe showed a positive 22q12 translocation in 1 of 2 FNA cases tested.
Conclusions: To our knowledge ours is the largest series describing the cytopathology of EMC. A confident cytologic diagnosis of EMC is dependent on the presence of a uniform, round to oval cell population arranged in cords and set in an abundant myxoid/chondromyxoid background, and arising in the appropriate clinical context. If positive, FISH testing (of paraffin cellblocks or smears) is confirmatory when coupled with the appropriate cytomorphology and clinical setting.

309 Quantitative Imaging Cytometry of Lymphoid Cytology Samples: Contribution of Cell Area, Texture Parameter and Relocalization to Classification

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Background: Stratification of lymphoid cytology samples into diagnostic categories is based on immunophenotyping by flow cytometry(FC) or quantitative imaging cytometry(QIC), cell size, chromatin texture and presence or absence of nucleoli. FC provides relative cell size only. QIC additionally provides a cell area (CA) calculation, ability to assess nucleoli and an index of nuclear texture. We investigated whether these features were useful in classification of hematolymphoid lesions in cytology samples.
Design: From over 1000 lymphoid cytology samples immunophenotyped by QIC using Clatch's method and CompuCyte™ instrumentation, cell size distribution was analyzed in 100 cases with ambiguous immunophenotype [follicular lymphoma Grade 1 or 2 (FL1-2) vs large B-cell lymphoma (LBCL) vs Burkitt lymphoma (BL)]. After immunophenotyping, unfixed cells were relocalized for visual examination using forward laser light scatter. In 20 cases the accuracy of the QIC calculation of CA was confirmed by image analysis (Image J) on photographs of unfixed, alcohol fixed and air dried cells. In 10 cases additional data provided by introduction of nuclear stains prior to relocalization was assessed.
Results: Image J and QIC calculation of CA corresponded closely when an identical plane of focus for photography was achieved. QIC measurement of CA of unfixed cells is about 10% greater than that of alcohol fixed cells and 10% less than that of air dried cells. CA values alone allowed stratification of FL1-2 (mean CA 66 μ^2) and BL from LBCL (mean CA 90μ^2) in 66% of cases. Variation of CA was significantly higher in LBCL than in FL1-2 and Burkitt Lymphoma. In cases where the CA of the clonally restricted population showed a "smear" overlapping small and large cell regions, the texture parameter based on forward light scatter was significantly higher in FL1-2 due to more condensed chromatin, and single large nucleoli could be visualized in unfixed cells in most LBCL. However confident visualization of smaller peripheral nucleoli in classical centroblasts and Burkitt lymphoma cells was only possible with use of a nuclear stain.
Conclusions: The calculated cell area, texture parameter and morphologic assessment possible when immunophenotyping lymphoid samples by QIC assists classification. Use of a nuclear dye permits resolution of small peripheral nucleoli allowing differentiation of centroblasts from large centrocytes, potentially providing a means of grading follicular lymphoma in lymph node aspirates by QIC.

310 The Clinical and Diagnostic Impact of Using a Standard Criteria of Adequacy Assessment and Diagnostic Terminology for FNA Diagnosis of Thyroid Nodules

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Background: Criteria for assessment of specimen adequacy and terminology (CAST) for cytological diagnosis of thyroid nodules vary among pathologists resulting in diagnostic inconsistency between pathologists and difficult communications with clinicians. A standard CAST was implemented in our institution since 2005 and was distributed and explained to all involved parties. This study was carried out to investigate the impact of using the standard CAST on FNA diagnosis and clinical management of thyroid nodules.
Design: The study compares FNAs performed 2 years prior to (G1) and 1.5 years after (G2) implementing the standard CAST . Specimens (Sp) include those with conventional smears (SM) made at on-site evaluation or those with ThinPrep(TP) only. The standard CAST defined Sp adequacy (ASp) and inadequacy (ISp) based on the presence and absence of at least 6 groups of follicular cells with minimal 10 cells in each group, respectively. Eight FNA diagnostic categories (CT) were established based on the PSC guidelines with minor modification. Diagnoses made for ASp without assigned CT were termed as descriptive diagnoses (DD). Cytology-histology correlation was studied for ASp with surgical follow-up. The parameters including non-diagnostic rate(ND%), diagnostic sensitivity(DS%), rate of DD(DD%), and rate of surgical follow-up(SF%) were calculated and analyzed using SigmaStat®.
Results:

Comparison of two periods prior to and after use of the standard CAST

PARAMETERS	G1	G2	P
Age of patients	49.6±0.6	52.6±0.6	<0.01
No. of Sp	764	824	-
SM/TP only (%)	41.2/58.5	64.0/36.0	<0.01
ND%	21.6	16.1	<0.01
DD%	14.5	3.7	<0.01
Non-neolplastic diagnosis%	68.3	77.3	<0.01
Neoplastic diagnosis%	12.8	10.7	>0.05
Follocular/Hurthle cell lesions%	4.3	7.4	<0.05
SF%	16.2	21.1	<0.05
SF%(non-neoplastc)	5.4	11.6	<0.01
SF%(neoplastic)	64.9	70.9	>0.05
DS%	63.9	75.0	>0.05

*P ≤0.05 is defined as level of statistical significance.

Conclusions: 1) Use of the standard CAST on FNA diagnosis of thyroid nodules significantly reduced ND% and DD%, providing more consistency among the pathologists as well as better communication between the pathologists and the clinicians. 2) While there is some change in trend among the categories, the DS% is not changed significantly. This may reflect the subjective component of assessment by the pathologists that may not be necessarily affected by CAST.

311 Is Direct Flourescent Antibody (DFA) Testing Needed in the Evaluation of Pneumocystis Carinii in Bronchoalveolar Lavage (BAL) Cytology Specimens?

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Background: DFA is often utilized in the evaluation of Pneumocystis carinii (PCP) in respiratory cytology specimens from immunocompromised patients. We evaluated the efficacy of DFA for PCP in bronchoalveolar lavage (BAL) specimens and compared the findings with Giemsa and GMS staining.
Design: Three hundred and sixty-four BALs were evaluated from 1/1/00 to 6/30/05. We compared the DFA results with the findings in Diff-Quik and GMS stained cytospin preparations, and determined the sensitivity of Diff-Quik, GMS and DFA studies.
Results: DFA was not performed in 128 patients, of which one also did not have GMS staining. Of the remaining 242 cases, 223 were negative for Diff-Quik, GMS and DFA studies. Eighteen cases were positive for PCP, including six positive for Diff-Quik, GMS and DFA; one positive for Diff-Quik and GMS with negative DFA; four Diff-Quik negative, GMS positive, DFA positive cases; two Diff-Quik negative, GMS positive, DFA negative and only one Diff-Quik and GMS negative, DFA positive case. The sensitivity of Diff-Quik, GMS and DFA was 60%, 93% and 79%, respectively.
Conclusions: We believe that routine DFA testing has little value for the diagnosis of Pneumocystis carinii in the cytologic work-up of BAL specimens from immunocompromised patients compared to conventional GMS staining, since GMS staining has greater sensitivity, more cost effective, less labor intensive and has a faster turn around time for expeditious reporting. However, DFA may have a selective role in GMS negative cases that are highly suspicious for Pneumocystis carinii.

312 Quantitative Methylation-Specific PCR for the Detection of Aberrant DNA Methylation of Tumor Suppressor Genes in High-Grade Squamous Intraepithelial Lesions (HSIL)

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Background: Current cervical cancer screening programs using the Papanicolaou (Pap) test are aimed at early detection and treatment of precancerous squamous intraepithelial lesions (SIL). Aberrant promoter methylation of selective tumor suppressor genes (TSGs) has been detected in SIL and invasive cervical cancers. Using real-time quantitative methylation-specific PCR (QMSP), the methylation profile of four TSGs (*DAPK1, IGSF4, SPARC,* and *TFPI2*) was determined and analyzed for the ability of each gene, and the genes in combination, to distinguish HSIL from low-grade SIL (LSIL) and negative liquid-based Pap tests.
Design: Genomic DNA from randomly selected biopsy-confirmed HSIL (n=39) and LSIL (n=30), and cytologically negative (NILM; n=30) residual liquid-based Pap tests was isolated and bisulfite-modified. Using QMSP, we analyzed the frequency and relative level of promoter methylation for *DAPK1, IGSF4, SPARC,* and *TFPI2*. The percentage of methylation (%M) for each TSG was calculated using the reference gene, *ACTB*, as the control for bisulfite conversion and total input DNA. The area under the receiver operating characteristic (ROC) curve (AUC) was used as a measure of test performance for the ability of TSG methylation to distinguish HSIL from combined LSIL/NILM samples. The cumulative methylation scores for each sample, defined as the sum of the %M of all 4 genes, were used to determine the AUC for the 4 TSGs in combination.
Results: For each TSG analyzed, significant differences in both frequency ($p \leq 0.0081$) and relative level ($p \leq 0.002$) of methylation were observed in HSIL compared to combined LSIL/NILM samples. Methylation of at least one TSG occurred in 64% of HSIL and 20% of LSIL/NILM samples ($p < 0.0001$). The cumulative methylation scores for the 4 TSGs were significantly higher in HSIL than in LSIL/NILM samples ($p < 0.0001$). Using AUC as a measure of test performance, methylation of each TSG had the ability to distinguish HSIL from combined LSIL/NILM samples (AUC range 0.6-0.672; $p \leq 0.0481$). The combination of 4 TSGs showed improved test performance (AUC=0.762; $p < 0.0001$).
Conclusions: Aberrant methylation of TSGs occurs more frequently in HSIL and at higher relative levels compared to LSIL/NILM Pap tests. ROC analysis demonstrates that methylation of each of 4 TSG analyzed can distinguish HSIL from combined LSIL/NILM samples. Analysis of 4 genes in combination improves test performance. Aberrant methylation of TSGs may function as a useful biomarker of HSIL in liquid-based Pap tests.

313 Glypican-3 Immunocytochemistry in Liver Fine Needle Aspirates: A Novel Stain To Assist in Differentiation of Benign and Malignant Liver Lesions

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Background: Glypican-3 (GPC3) is a heparan sulfate proteoglycan bound to the cell surface by a lipid anchor, regulating activity of certain cytokines, such as Wnts. Recent studies have shown that serum GPC3 levels are significantly increased in hepatocellular carcinoma (HCC) patients, but are undetectable in healthy liver donors and patients with benign liver disease. GPC3 has proven to be a promising marker of HCC in histological sections by immunohistochemistry. To our knowledge, in this study, GPC3 protein expression is explored for the first time, in liver fine needle aspirates (FNAs) by immunocytochemistry.

Design: Archival direct smear and Cytolyt-fixed (Cytyc, Boxboro, MA) material from hepatic FNAs at two institutions were retrieved; these comprised 60 slides from 20 cases of proven HCC (group A), 20 cases of metastatic adenocarcinoma to the liver (group B) and 20 cases with benign hepatocytes (group C). Following blockage of endogenous peroxidase and protein, antigen retrieval was performed. Primary antibody GPC3 (Clone 1G12, BioMosaics Inc, Burlington, VT), and secondary antibody Mouse Envision Polymer (DakoCytomation, Dako Corporation, USA) were used, followed by DAB chromogen (DakoCytomation). The slides were counterstained with hematoxylin, and examined by 5 observers. GPC3 staining intensity and pattern were graded by a 4-tier system as: negative=0, weak cytoplasmic=1, moderate cytoplasmic=2, and strong cytoplasmic with membranous accentuation=3.

Results: GPC3 immunoreactivity was cytoplasmic with membranous, and occasionally perinuclear, accentuation. Group A had stronger immunoreactivity compared with groups B and C. Group A (HCCs) showed 10% grade 0-1 and 90% grade 2-3 staining. In contrast, group B (metastatic adenocarcinoma) and C (benign hepatocytes) resulted in 100% grade 0-1 and 0% at grade 2-3. Sensitivity and specificity at grades 2-3 were 90% and 100% respectively, $p < 0.001$.

Conclusions: In this study, 90% of HCC in archival liver FNAs were strongly positive for GPC3, with cytoplasmic accentuation present in 70%. All benign hepatocytes and metastatic adenocarcinomas were negative or stained only weakly for GPC3. Our data supports the potentially significant diagnostic utility of GPC3 in FNAs as a reliable tool in differentiating benign from malignant liver lesions, and primary liver tumors from metastases to the liver.

314 What Can We Learn from High Risk HPV (HR-HPV) Positivity among Cases Upgraded or Downgraded to Atypical Squamous Cells of Undetermined Significance (ASC-US) by the Pathologist?

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Background: Pathologist rescreening of Pap tests interpreted as Negative for Intraepithelial Lesion or Malignancy (NILM) results in some upgrades to ASC-US. Similarly, a proportion of Pap tests interpreted as Squamous Intraepithelial Lesion (SIL) are downgraded to ASC-US. This study was undertaken to determine the HR-HPV positivity rate among upgraded and downgraded cases to ASC-US.

Design: At our institution, reflex testing for HR-HPV is compulsory for liquid based pap tests (LBPT) interpreted as ASC-US. All reports with the final diagnosis of ASC-US during the time period from 7/1/03 to 7/31/06 were retrospectively reviewed to identify cases upgraded from NILM to ASC-US and downgraded from SIL to ASC-US by the pathologist. HR-HPV positivity rates in the overall ASC-US category, the upgraded cases, and the downgraded cases were determined for the department and for each pathologist. Our database was searched for histologic follow-up for all upgraded and downgraded cases testing positive for HR-HPV.

Results: During the time period of study, 8964 ThinPrep® LBPT were evaluated resulting in 337 ASC-US cases which were tested for HR-HPV using the Digene Hybrid Capture® II HPV test. ASC-US interpretations were the result of an upgrade in 21.2% (72/337) of cases and the result of a downgrade by the pathologist in 6.5% (22/337) of cases. The overall and individual pathologist profiles regarding HR-HPV positivity are presented in table 1.

HR HPV positivity rates

	MD #1	MD #2	MD #3	MD #4	LAB TOTAL
% positive overall (HPV+/total)	50.0% (41/82)	38.4% (56/146)	55.8% (29/52)	31.6% (18/57)	42.7% (144/337)
% positive in Upgrades (HPV+/total)	28.6% (2/7)	23.5% (8/34)	63.6% (7/11)	40.0% (8/20)	34.7% (25/72)
% positive in Downgrades (HPV+/total)	75.0% (6/8)	28.6% (2/7)	60.0% (3/5)	50.0% (1/2)	54.5% (12/22)

Histologic follow-up was available in 10 upgraded cases (6 negative, 3 CIN I, 1 CIN III) and in 6 downgraded cases (4 negative, 2 CIN I).

Conclusions: Our results support the importance of rescreening as part of quality assurance. Additionally, they suggest that tracking HR-HPV positivity rate among upgraded cases may be used as an indicator for the quality of screening. Individual pathologist profiles regarding HR-HPV positivity rate in both upgraded and downgraded cases may serve as an additional tool in adjusting one's threshold when diagnosing ASC-US.

315 The Utility of Fluorescence In Situ Hybridization for the Detection of Urothelial Carcinoma in Residual Urine Cytology Specimens with an Equivocal Diagnosis

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Background: Urine cytology and cystoscopy are techniques used for detecting and monitoring patients for urothelial carcinoma (UC). The clinical management of patients with equivocal (atypical, suspicious, etc.) urine cytology diagnoses in the absence of clinically detectable tumor can be challenging since these patients often have, or ultimately develop UC. Fluorescence in situ hybridization (FISH) is a novel technique that utilizes fluorescently-labeled DNA probes to detect urinary cells with chromosomal abnormalities. The goal of this study was to determine the utility of FISH in the management of patients with an equivocal cytology diagnosis.

Design: Ninety-three residual urine samples from 74 males and 19 females (age 46-89 years; mean, 71 years) with a cytology diagnosis of atypical (N=50) or suspicious (N=43), same day cystoscopy result, and bladder biopsy within three months of the cytology diagnosis were collected and processed for FISH analysis. The FISH UroVysion™ probe set was used and specimens were considered positive by FISH if ≥ 4 cells demonstrated gains of two or more signals (polysomy) or if ≥ 20% of cells demonstrated homozygous 9p21 deletion.

Results: Forty-three (46%) of the 93 specimens analyzed were diagnosed as positive for malignancy by FISH (39 polysomy, 4 homozygous 9p21 deletion). Thirty-nine (91%) of these 43 patients had pathologic evidence of UC (10 pTa, 11 CIS, 2 T1, and 16 muscle-invasive tumors). Of the 4 remaining patients with a positive FISH result and negative biopsy, 1 patient had CIS on a follow-up biopsy, 1 had a follow-up positive cytology result, and the other two currently have no evidence of malignancy (follow-up time, 0 and 4 months). Fifty specimens were diagnosed as negative by FISH. Twenty-nine (58%) of these patients had negative biopsy results whereas the remaining 21 (42%) demonstrated biopsy proven UC staged as pTa (15), CIS (3), T1 (1), and T2 (2) tumors. FISH and cystoscopy alone detected 39/60 (65%) and 38/60 (63%) of cancers, respectively. When combined, 54/60 (90%) of patients with biopsy proven UC were identified. The six undetected tumors by both methodologies included 1pTaG1, 2pTaG2, 2 CIS, and 1 muscle-invasive UC with squamous differentiation.

Conclusions: The data from this study suggests that FISH, in conjunction with cystoscopy, may aid clinicians in the diagnosis of UC in patients with an equivocal cytology diagnosis.

316 The Diagnostic Role of Claudins in Serous Effusions

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Background: The aim of this study was to analyze the diagnostic role of claudins, integral membrane proteins of tight junctions, in effusion cytology.

Design: Three hundred and twenty-five effusion specimens, consisting of 218 ovarian, 49 breast, 15 cervical/endometrial, 10 gastrointestinal and 8 lung adenocarcinomas (AC) and 25 malignant mesotheliomas (MM) were analyzed for claudin-1 and claudin-3 expression using immunohistochemistry. Ovarian (218) and breast (26) AC were further analyzed for claudin-7 expression.

Results: Claudin-1 membrane expression was most frequent in metastatic ovarian, cervical and endometrial AC, with significantly less expression in AC of other origin and malignant MM ($p<0.001$, Kruskal-Wallis H Test). Claudin-3 membrane expression was comparable in AC of different origins, but was essentially negative (1/25 specimens with <5% expression, 24 negative) in MM ($p<0.001$). Reactive mesothelial cells (RMC) in AC effusions were focally (<5% expression) positive for claudin-1 and claudin-3 in only 6/110 and 3/110 specimens, respectively. Claudin-7 membrane expression was significantly higher in ovarian compared to breast AC ($p<0.001$).

Conclusions: Our data support a role for claudin-3 as a new diagnostic marker for the differential diagnosis between metastatic AC, MM and RMC in effusions, with a similar role for claudin-1 in the differentiation between AC and RMC. Claudin-1 and claudin-7 may be useful in a panel of markers for determining the origin of metastatic AC in effusions.

317 Low Grade Squamous Intraepithelial Lesion (LSIL) and High Risk HPV Status: Effect of Age on Follow-Up with High Grade Cervical Lesions

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Background: Current guidelines for LSIL management require colposcopic examination due to high rates of high-risk human papillomavirus (HPV) association. However, age stratification data may be important in modifying these guidelines. The present study analyzes HPV status by age in cases of LSIL in our laboratory.

Design: All LSIL cases with an HPV test over a 2 year period were analyzed by patient age, HPV result, and for followup biopsy results. Biopsy results were included if taken up to 1 year after the initial cytology sample was collected.

Results: There were 245 LSIL specimens with HPV results, and 168 had followup biopsies. Patient ages ranged from 15-77 years. 82.0% of all cases were HPV+. HPV+ rates were 83.9% for patients <30, and 79.4% for ≥30. Grouping ages by decade, HPV+ rates ranged from 65.2%-100%. HPV+ rates were 89.7% in patients <20, and were approximately 80% for most other age groups. (Table 1) 16.1% of all LSIL cases had a CIN2+ followup biopsy. The rate increased to 19.0% in patients <30, and decreased to 11.8% in ≥30. For all ages, HPV had a sensitivity for CIN2+ of 92.6% and a NPV of 92.0%. For patients <30, the sensitivity and NPV were 100%. For patients ≥30, the sensitivity was 75% and the NPV was 83.3%. There were two false negative HPV results (CIN2-age 37, CIN3-age 34) out of 25 HPV- cases. (Table 2)

Conclusions: The HPV+ LSIL rate in this study is similar to that of the ALTS trial (82.9%). The 16.1% chance of harboring CIN2+ with an LSIL result is also similar to the ALTS finding of 15%. After a negative HPV test, this chance decreases to 8%. Patients <30 have a higher rate of CIN2+ (19.0%) compared to ≥30 (11.8%). In ages <30, the risk of CIN2+ after an HPV- LSIL was 0%. For ages ≥30, the risk of CIN2+ after an HPV-LSIL increased from 11.8% to 16.7%, which is likely due to the small sample size and because the 2 false negative cases involved patients in their mid-30s. Overall, it appears that a negative HPV test reduces the chance of subsequent CIN2+ in patients <30, and for the studied population as a whole. Though some of the findings are supportive of new management guidelines for LSIL, additional research may be needed.

Table 1: HPV+ Rate by Age

Age	# Specimen (% of all ages)	# HPV+ (% within age group)
<30	143 (58.4%)	120 (83.9%)
≥30	102 (41.6%)	81 (79.4%)
All ages	245 (100.0%)	201 (82.0%)

Table 2: Biopsy Diagnosis Following HPV- LSIL by Age

Age	# HPV- specimen	CIN2+	CIN1	NILM
<30	13	0	5	8
≥30	12	2	2	8
All ages	25	2	7	16

318 Cervical Mircroglandular Hyperplasia and Squamous Metapalsia Have a Higher Chance of Being Interpreted as Abnormal in Cervical Smears

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Background: Microglandular hyperplasia (MGH) is a benign endocervical proliferation with wide cytomorphological patterns. This may lead to abnormal interpretations. Some studies reported previously have evaluated the cytomorphological spectrum of MGH in conventional cervical smears. Cytological features in biopsy-proven MGH cases and its impact on interpretation of liquid-based preparations has not been reported.

Design: Cervical biopsies without dysplasia but with four types of histologic interpretations [A. MGH (19); B. MGH with HPV (31); C. Squamous metaplasia (36); D. Negative (21)] were selected. Cases with Liquid-based cervical smears (Sure-Path™, TriPath) of the respective cases obtained at the time of surgical biopsy or within the preceding 3 months were evaluated. Cytopathologic interpretations reported initially were statistically analysed.

Results:

Cytopathologic interpretation pattern associated with each group.

Biopsy	Cytopathologic interpretations					
results	Negative	Abnormal				
		ASCUS	AGUS	LSIL	ASCH	Total
A) MGH without HPV (p<0.20)*	61%(19/31)	6%(2/31)	10%(3/31)	19%(6/31)	4%(1/31)	39%(12/31)
B)MGH with HPV (p<0.001)*	5%(1/19)	37%(7/19)	11%(2/19)	42%(8/19)	5%(1/19)	95%(18/19)
C) Squamous metaplasia (p<0.001)*	19%(7/36)	33%(12/36)	0	47%(17/36)	0	81%(29/36)
D) Negative*	95%(20/21)	0	0	5%(1/21)	0	5%(1/21)

*p values were calculated by comparing with negative group by Chi-Square test.

Conclusions: As compared to negative group, MGH with HPV and squamous metaplasia were associated with statistically significantly higher number of abnormal cytologic interpretations. Similar to MGH, squamous metaplasia had higher probability of being reported as ASCUS or LSIL. However, MGH (with or without HPV) was associated with significantly increased AGUS and ASC-H interpretations.

319 Atypical Urine Cytology on ThinPrep: Correlation with Fluorescence *In Situ* Hybridization and Histology

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Background: Urine cytology (UrCyt) and Fluorescence *in situ* hybridization [FISH (UroVysion, Vysis Inc., Dover's Grove, IL)] are being used in conjunction for primary detection and surveillance of urothelial carcinoma (UCA). The correlation of atypical UrCyt (Aty UrCyt) and FISH with follow-up biopsy (BX) has not been fully elucidated.

Design: Data for Aty and malignant (UCA) UrCyt and concurrent FISH over a 27-month-period (Jan' 02 - April' 04) was reviewed and correlated histologically with BX taken within a month of the latter two tests. All UrCyt was one Papanicolaou-stained ThinPrep (TP) slide. FISH was categorized as negative (Neg) and positive (Pos).

Results: Of 564 UrCyt specimens 64 were diagnosed as: Aty, 35 and UCA, 29. FISH was: Neg, 19; Pos, 45. BX was: Neg, 30; UCA, 34 [low-grade (LG), 20 and high-grade (HG), 14]. Table 1 shows the distribution of Aty & UCA UrCyt, FISH and BX. Aty UrCyt and Pos FISH detected 14/16 (87.5%) UCA and missed 2/16 (12.5%) UCA, both LGUCA (p= 0.0023)

Distribution of Urine Cytology, FISH & Biopsy Results

Aty UrCyt & FISH	Neg BX	BX-LGUC	BX-HGUC	Total
Aty UrCyt & Pos FISH	8 (36.36%)	9 (40.9%)	5 (22.72%)	22 (62.85%)
Atyp UrCyt & Neg FISH	11(84.61%)	2 (15.38%)	0 (0%)	13 (37.14%)
Total	19 (54.28%)	11(31.42%)	5 (14.28%)	35 (100%)
Pos UrCyt & FISH	Neg BX	BX-LGUC	BX-HGUC	Total
Pos UrCyt & Pos FISH	8 (34.8%)	6 (20.08%)	9 (39.13%)	23 (79.31%)
Pos UrCyt & Neg FISH	3 (50%)	3 (50%)	0 (0%)	6 (20.68%)
Total	11(37.93%)	9 (31.03%)	9 (31.03%)	29 (100%)

Table 1

Table 2 shows the data on FISH with BX. Pos FISH correctly detected 29/34 (85.3%) of UCA (LGUCA, 15; HGUCA, 14) and missed 5/34 (14.7%) of UCA, all LGUCA (p=0.0024).

Distribution of FISH and BX

	BX Neg	BX Pos	Total
FISH Pos	16 (35.5%)	29 (64.4%)	45 (70.3%)
FISH Neg	14 (73.7%)	5 (25%)	19 (29.7%)
Total	30 (46.9%)	34 (53.1%)	64 (100%)

Table 2

Conclusions: 1) Atypical cells in urine cytology by themselves cannot be definitely categorized as benign or malignant. 2) When combined with FISH, Aty UrCyt tends to have increased sensitivity for detection of UCA. 3) Sensitivity of FISH assay supersedes that of cytology in detecting UCA.

320 A Prospective Study of Frozen Section and Rapid Cytokeratin Immunostaining for the Intraoperative Evaluation of Axillary Sentinel Lymph Nodes in Breast Cancer

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Background: Intraoperative evaluation of axillary sentinel lymph nodes (SLNs) allows the surgeon to complete axillary dissection in one setting if they are positive. There is no consensus regarding the optimal method for intraoperative evaluation of SLNs. We compared frozen section (FS) and rapid cytokeratin immunostaining (RCI) with touch preparation (TP) for intraoperative evaluation and compared the result with final pathological examination (FP) of the axillary SLNs.

Design: We included 96 patients with invasive breast carcinomas who underwent SLN biopsy. TP and FS were performed on all SLNs which were stained with hematoxylin and eosin. RCI was performed using EPOS cytokeratin (DAKO), which was standardized to be completed in 25 minutes. The results of FS and RCI were compared with TP and FP of SLNs including one H&E stain and one CK immunostain of the third level.

Results: The 96 cases consisted of 88 cases of invasive ductal carcinomas, 6 invasive lobular carcinomas, 1 adenoid cystic carcinoma and 1 metaplastic carcinoma. Twenty tumors were less than 1 cm in size, 62 measured 1-2 cm, 12 between 2-3 cm and 2 cases were more than 3 cm. Metastatic carcinoma was detected in SLNs by one or another method (TP, FS, RCI, FP) in 17 patients (18%), all of whom had invasive ductal carcinomas (5 were less than 1 cm, 10 were 1-2 cm, and 2 were larger than 2 cm); 10 of these were macrometastases (2mm to 1.0 cm) and 7 were micrometastases (0.1mm to 2 mm). TP detected 7/10, FS 9/10, RCI 9/10 and FP 10/10 of the macrometastases. TP detected 1/7, FS 4/7, RCI 5/7 and FP 5/7 of the micrometastases. The sensitivity was 47% for TP, 76% for FS, 82% for RCI, 82% for FS+RCI and 88% for FP. The false negative rate was 53% for TP, 24.0% for FS, 18% for RCI, 18% for FS+RCI and 12% for FP.

Conclusions: 1. FS plus RCI of axillary SLNs detected metastatic carcinomas in SLN at comparable rate as FP (sensitivity of 82% vs 88%). 2. The ability to measure the size of a metastatic carcinoma intraoperatively by using FS and RCI may be useful in the decision making process for completion of axillary dissection. 3. Compared with TP, FS plus RCI offers increased sensitivity and the ability to ascertain the size of metastatic carcinomas in the lymph node during the intraoperative evaluation of axillary SLNs in breast cancer. 4. The increased sensitivity of FS plus RCI was due mainly to the increased detection of micrometastases.

321 Carbonic Anhydrase IX (CAIX) and Human Papillomavirus (HPV) as Potential Diagnostic Biomarkers of Cervical Dysplasia/Neoplasia in Women with a Cytologic Diagnosis of Atypical Glandular Cells of Undetermined Significance (AGUS): A Gynecologic Oncology Group (GOG) Study

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Background: The lack of well-established criteria to separate reactive glandular atypia from neoplasia contributes to the challenge of managing women with a cytologic diagnosis of AGUS. Women with AGUS are often found to have a significant cervical lesion including high grade dysplasia (CIN 2, CIN 3), adenocarcinoma in situ (AIS) or invasive carcinoma (CA). CAIX is a marker of hypoxia that is expressed at high levels in cervical neoplasia but not in benign lesions. The GOG initiated a group-wide trial to evaluate expression of CAIX in a Pap smear (PS) as a potential diagnostic biomarker of cervical neoplasia and subsequently amended the study to test for HPV in a liquid based specimen.

Design: Patients with a cytologic diagnosis of AGUS and a satisfactory PS who underwent a complete histologic evaluation of the cervix within 6 months of diagnosis were eligible for the study. The PS immunostained with CAIX was evaluated by SYL, WHR, and TB as positive/negative. Hybrid Capture 2 testing was used to detect the presence of high-risk HPV DNA. Histologic diagnosis was determined centrally by the GOG Pathology Committee.

Results: 684 patients were enrolled on the study. Of the 528 eligible women entered on study, 160 had significant lesions: CIN2, CIN3, AIS or CA, 503 were tested for CAIX, and 219 were tested for HPV. The overall sensitivity for cervical significant lesions was 73% for CAIX, 78% for HPV, and 93% for CAIX + HPV. When categorized as glandular lesions (GL) or squamous lesions (SL), the sensitivity for SL vs GL was 63 vs 89% for CAIX, 90 vs 63% for HPV, and 93 vs 93% for CAIX + HPV, respectively. The false-negative rate was 13% for CAIX, 12% for HPV, and 6% for CAIX + HPV.

Conclusions: Combining CAIX with HPV improved the diagnostic accuracy of cervical squamous and glandular lesions in women with a cytologic diagnosis of AGUS. Additional biomarkers will be evaluated alone and in combination with CAIX and HPV to further improve diagnostic accuracy and reduce the false negative rate below 5%.

322 Diagnostic Utility of the Immunocytochemical Expression of Glypican-3 Oncofetal Protein in Fine Needle Aspiration Biopsies of Hepatic Lesions in Differentiating Hepatocellular Carcinoma from Other Primary and Metastatic Hepatic Lesions

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Background: Glypican-3 (GPC3), a heparin sulfate proteoglycan anchored to the cell membrane, is expressed in tissue obtained from hepatoblastomas and hepatocellular carcinomas (HCCs) in 84% of the cases but in none of hepatic adenomas, focal nodular hyperplasia (FNH), cholangicarcinomas or the majority of metastatic lesions to the liver. We evaluated the immunocytochemical (ICC) expression of GPC3 in archival material obtained from fine needle aspiration (FNA) of hepatic lesions to assess the sensitivity, specificity, positive predictive value (PPV) and the negative predictive value (NPV) of GPC3 in alcohol-fixed cytological material, and evaluate its potential diagnostic utility in differentiating primary malignant hepatocellular lesions from benign hepatic lesions and from other malignant tumors metastatic to the liver.

Design: Forty-nine FNAs of the liver obtained between January 2000 and June 2006 were selected from our files. Cytologic diagnoses (confirmed by tissue diagnosis and/or clinical follow-up) included: 7 adenomas, 1 FNH, 24 HCCs, and 17 metastatic tumors. These cases consisted of alcohol-fixed Papanicolaou stained slides that were stained with a mAb to Glypican-3 (1G12 clone, Biomosaics Inc, Burlington, VT). Results were recorded as positive or negative. The cytoplasmic reactivity was either diffuse or focal, and the intensity was strong or weak.

Results: Based on histological, clinical, and/or radiological follow up, 20 of 24 (83.3%) of FNAs confirmed positive for HCC expressed GPC3. All 7 adenomas and the only FNH did not express GPC3. 16/17 aspirates from metastatic malignancies: colon (0/3), pancreas (0/3), breast (0/2), gastric (0/2), adenoid cystic carcinoma (0/1), lung small cell (0/1), non-small cell carcinomas (0/3), and one sarcoma (0/1), were all negative for GPC3. The only case expressing GCP3 was a carcinoma of unknown origin. The sensitivity, specificity, PPV, and the NPV of GPC 3 in the diagnosis of HCC in FNAs were as follows: Sensitivity 83.3%, Specificity 96%, PPV 95%, and NPV 85.7%.

Conclusions: ICC staining for GPC3 in alcohol-fixed FNA material is a highly sensitive and specific method able to differentiate HCC from other benign and malignant hepatic lesions, and most metastatic lesions.

323 Cost-Effective Diagnostic Use of Immunohistochemical Stains in Identifying Malignant Cells in Effusion

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Background: Malignant effusion represents a small percentage of body fluid specimens received in pathology department. A study was undertaken to determine the cost-effective use of immunohistochemical stains.

Design: A retrospectic review of all body fluid specimens was performed to identify cases positive for malignant cells and the minimal numbers of immunostain markers needed. Pap stain and cell block section slides were reviewed in all cases. For initial evaluation, our department used 3 well-characterized immunostain markers (BerEp4, CEA, CD15[LeuM1]), chosen for their sensitivity and relative specificity. When neutrophils were abundant, B72.3 was used instead of CD15. Secondary antibodies were used to determine the possible sites of origin in positive cases. Mesothelial markers were used when clinical suspicion of mesothelioma was raised.

Results: In a period of 25 months, 1028 cases of effusion were received, 41 cases were further evaluated using immunostains. 10 cases were frankly malignant and confirmed by the stains (2 in the lung, 5 from breast, 3 non-epithelial malignancy). Remaining cases (31), 24 were positive for malignancy and 7 were benign reactive changes. Positive cases were confirmed by surgical exploration or image studies. The malignant tumors were from lung (10), breast (4), ovary (6), pancreas (3) and prostate (1). The positive immunostain results: BerEp4 23/24, CEA 18/23, CD15 10/11, B72.3 5/7. 24/24 cases were positive for 1 marker, 20/24 were positive for 2 markers, and 7/19 were positive for 3 markers. No mesotheliomas were identified in the study period.

Conclusions: In this study using 3 epithelial markers (BerEp4, CEA, and CD15/B72.3), 2 positive markers predicted malignancy in 20/24 cases, and 1 positive marker identified all positive cases. BerEp4 provided the most reliable results and is the most widely used marker in differentiaing carcinoma from mesothelioma. Although benign mesothelial cells may show positive stain in any of the markers used, our case number is not large and there were no false positive cases. Methotheliomas were very rare (0 case in the study period). Our institute identified 3 cases in 10 years period, all were clinically evident (diffuse mass lesion in the pleural / peritoneal cavity). Benign reactive mesothelial cells were negative for the 3 epithleial markers (7/7). The use of 3 epithelial markers, including BerEp4 can successfully separate carcinoma from benign reactive mesothelial cells in the great majority of effusion specimens.

324 Efficacy of Molecular Studies in Esophageal Brushing Cytology in Patients with Barrett's Esophagus

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Background: Esophageal brushing cytology (EBC) for the evaluation of Barrett's esophagus (BE) has been used to guide surveillance and treatment. BE with dysplasia is a precancerous lesion that needs to be either closely followed for low grade dysplasia or treated for high grade dysplasia. However, separation of BE with dysplasia from BE with reactive/reparative atypia in EBC can occasionally be very challenging. Using quantitative methods applied to microdissected cell clusters from the smears selected based on cytologic features, we investigated the efficacy of determining molecular studies in separating reactive/reparative atypia from dysplasia and dysplasia from adenocarcinoma in EBC.

Design: Thirty-four EBCs were retrieved and divided into 4 groups as shown in Table 1. Representative cells were microdissected from cytology slides under stereoscopic guidance and DNA from these cells were extracted. LOH was quantitatively determined for a broad panel of 17 microsatellite repeat markers near tumor suppressor genes by PCR with labeled oligonucleotides followed by automated capillary electrophoresis.

Results:

Table 1. Genetic Mutation Accumulation in EBC

Group	No.	Ranges of Mutations	Average ± SD Mutations	P Values**
Normal Mucosa	10	0 - 1	0.60 ± 0.52	
IM without dysplasia	11	0 - 5	2.03 ± 1.64	P1 = 0.014
IM with dysplasia	7	3 - 6	4.71 ± 1.11	P1 = 0.00002, P2 = 0.001
Adenocarcinoma	6	5 - 8	6.50 ± 1.22	P1 = 0.00003, P2 = 0.00003, P3 = 0.02

*: IM: Intestinal metaplasia. **: $P < 0.05$, significant difference; $P < 0.01$, very significant difference.

Our data showed that 1 to 3 mutations was often associated with IM without dysplasia, more than 3 mutations but less than 6 mutations associated with IM with dysplasia, and 6 or more mutations associated with adenocarcinoma.

Conclusions: There is accumulation of LOH from reactive/reparative atypia to malignancy with statistically significant differences between BE with reactive atypia from dysplasia and dysplasia from carcinoma in EBC. We believe that EBC examination with molecular studies can be helpful in distinguishing BE with reactive/reparative atypia from dysplasia, and dysplasia from carcinoma in problematic cytology cases and thereby help select patients for surveillance and treatment.

325 The Clinical Utility of Endocervical Curettage (ECC) for Patients with Persistent ASCUS Pap Smears but a Negative Colposcopic Examination: A Correlative Cytologic and Histologic Study of 502 Cases

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Background: In Bethesda system terminology, *Atypical Squamous Cells of Undetermined Significance (ASCUS*) refers to a cytologic abnormality that exhibits insufficient atypia to constitute a squamous intraepithelial lesion (SIL). Current clinical guidelines recommend that patients (pts) with persistent ASCUS smears undergo colposcopic exam, sometimes with concomitant ECC. The aim of this study is to evaluate the utility of ECC in the detection of SILs in patients with persistent ASCUS smears but negative colposcopy, since the role of ECC is presently unclear.

Design: 502 ECC cases from pts who had received 3 or more ASCUS diagnoses were retrieved from our institutional pathology archives over a 6 year period (2000-06). Cases were identified by a solo ECC specimen, without a biopsy, but with multiple prior ASCUS Pap smears, reflecting a negative or unsatisfactory colposcopic examination. The pts age, specimen adequacy, presence of squamous epithelium, type of SIL, and the status of high risk HPV (HR-HPV) DNA testing were recorded.

Results: Of 502 ECCs, 61 cases (12.2%) were only composed of mucus and were insufficient for pathologic diagnosis; 312 cases (62.2%) showed only normal endocervical glands insufficient for evaluating squamous lesions; and 112 cases (22.3%) contained normal squamous epithelium. Low-grade SIL (LSIL) was present in 13 of 502 (2.6%) ECCs, whereas high-grade SIL (HSIL) was present in 4 of 502 (0.8%) ECCs. Pts ages ranged from 16 to 79 yrs (mean= 33.9 yrs). 13 pts with LSIL in an ECC ranged from 21 to 50 yrs (mean = 36 yrs). HSIL pts were 44, 47, 75 and 79 yrs old, respectively. Of 502 pts, 438 underwent HPV DNA testing and 317 cases (72.4%) tested positive for one or more HR-HPV serotypes, whereas 121 cases (27.6%) tested negative for HR-HPV. 11 of 13 (84.6%) pts with LSIL in their ECC and 4 of 4 (100%) pts with HSIL in their ECC tested positive for HR-HPV.

Conclusions: 1) ECC may be a suboptimal tool in managing women with persistent ASCUS but negative colposcopic examination, particularly since the majority of specimens contain either no to scanty tissue, or only normal endocervical glandular epithelium with no squamous epithelium. 2) ECCs in women < 40 years old with ASCUS but negative colposcopy seldom show HSIL, but sometimes an LSIL. 3) ECC is useful for women > 40 years old with ASCUS and positive HR-HPV testing, even if the colposcopic exam is negative.

326 Immunodetection of Squamous Intraepithelial Lesions Using a Cocktail of p16 and ProEXC in Liquid-Based Cytology Specimens on Cell Block Sections

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Background: Our previous study showed p16 to be a highly sensitive marker in confirming the diagnosis of high-grade squamous intraepithelial lesion (HGSIL), but it exhibited relatively low sensitivity for low-grade squamous intraepithelial lesion (LGSIL) in liquid-based cytology specimens on cell block sections (CBS). To increase diagnostic sensitivity in identifying LGSIL, we demonstrated that a cocktail of p16 and ProEXC (a mixture of MCM2 and DNA topoisomerase IIA) could detect 100% of LGSIL in cervical biopsy specimens. To improve diagnostic sensitivity for LGSIL in Pap smears, we evaluated the same cocktail in liquid-based cytology specimens on CBS.

Design: Forty-eight liquid-based cytology specimens were prepared for cell block (CB). Three categories of cases were included in this study: Group #1 (G1) – 17 cases of LGSIL; Group #2 (G2) – 15 cases of HGSIL; and Group #3 (G3) – 16 negative/reactive cases. The diagnoses in all cases in G1 and G2 were confirmed by cervical biopsy. Immunostaining with monoclonal antibodies against p16 (Dako) and a cocktail of p16 and ProEXC (TriPath) were performed on the formalin-fixed, paraffin-embedded CBS. The results were recorded as negative (no staining) or positive (>3 atypical squamous cells, with both cytoplasmic and nuclear staining or nuclear staining).

Results: Four slides with adequate cellularity were obtained from each CB. The results are summarized in Table 1. In G1, 13 of 17 cases were positive for p16. All cases in G1 were positive for the p16/ProEXC cocktail, including the 4 p16-negative cases. In G2, all 15 cases were positive for both p16 and the p16/ProEXC cocktail. In G3, 2 of 16 cases (10.5%) showed false immunoreactivity for both p16 and the p16/ProEXC cocktail. Most of the positively stained cells in G3 were metaplastic squamous cells or endocervical cells.

Table 1. Comparison of Positive Immunostaining Results in Three Groups

Marker	G1 (%)	G2 (%)	G3 (%)
p16	13/17 (76%)	15/15 (100%)	2/16 (10.5%)
p16 + ProEXC	17/17 (100%)	15/15 (100%)	2/16 (10.5%)

Conclusions: Our preliminary data indicate that a cocktail of p16 and ProEXC provides higher diagnostic sensitivity (100%) for both LGSIL and HGSIL than p16 alone and can potentially serve as a novel marker for screening squamous intraepithelial lesions in liquid-based cytology specimens on CBS. However, caution should be exercised because metaplastic squamous cells and endocervical cells may react positively as well.

327 Fine Needle Aspiration of the Thyroid Gland: Clinical Features and Implications of Indeterminate Diagnoses

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Background: Fine-needle aspiration (FNA) is a vital tool in the diagnosis and treatment planning for thyroid nodules. An indeterminate category in cytology is usually comprised of diagnoses such as follicular lesion, atypical cytology, and suspicious for malignancy. The clinical features may be useful in stratifying the risk of malignancy in some of these cases.

Design: Thyroid FNA and histologic follow-up between January 2000 and December 2005 from one institution were retrospectively reviewed. FNA diagnoses were traditionally categorized as positive, negative, indeterminate for malignancy, or unsatisfactory for diagnosis. The indeterminate cases were subclassified as follicular lesion (FL), atypical cytology cannot rule out papillary carcinoma (AC), and suspicious for malignancy (SFM). The diagnosis of FL was made when the aspirate consisted of microfollicles without colloid. The diagnosis of AC was used when some features of papillary carcinoma, including nuclear grooves, fine nuclear chromatin, and nucleoli, were focally observed, whereas SFM was characterized by the presence of rare intranuclear pseudoinclusions. Chi-square analysis was used to determine statistical significance between groups.

Results: Histopathologic diagnosis was available on 343 cases, including 193 cases of FL, 116 of AC, and 34 of SFM. Thyroid carcinomas were found in 22% (42/193) of FL, 37% (43/116) of AC, and 59% (20/34) of SFM diagnoses ($p<0.001$). Papillary carcinoma and its variants comprised 90% of all malignancies. The most common variant of papillary carcinoma found on histology was the occult (microscopic) variant (36%), followed by the follicular (25%) and classical variants (23%). The rate of malignancy was not significantly different between men and women ($p=0.99$). The rate of malignancy was 19% and 33% in African Americans and Caucasians, respectively ($p=0.026$). Patients younger than 50 years of age were more likely to have malignant lesions than patients 50 years and older (39% vs. 23%, $p=0.0014$).

Conclusions: A diagnosis of follicular lesion does not carry the same implications as those diagnosed with atypical cytology or suspicious for malignancy. When the cytologic findings present on FNA is indeterminate for malignancy, clinical features such as the patient's age and race, but not sex, would be helpful in the risk stratification and treatment planning in this patient population.

328 Cytology Versus DNA Ploidy in the Prediction of Persistence or Recurrence of Urothelial Cell Carcinoma (UCC)

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Background: Routine cytology is widely used for monitoring UCC patients in the detection of disease persistence or recurrence, but urothelial cytology reports are often not definitive. This study compared the accuracy of cytology and DNA ploidy status to predict biopsy and resection results in patients with UCC.

Design: 108 urinary bladder washing samples from 71 patients biopsy proven UCC were evaluated by both Papanicolou stained conventional cytology using the ThinPrep processing technique (Cytyc, Inc., Marlboro, MA) and for DNA content on Feulgen stained aliquots using the CAS 200 Image Analyzer. The clinical observation period ranged from 3 to 238 months. Cytology results were classified either as positive or non-positive (includes negative or atypical). A non-diploid (aneuploid) histogram was defined as either a DNA index of greater than 1.23, a tetraploid peak greater 25% of total cells or the presence of multiple polyploid peaks. Corresponding tumor biopsies or resections were classified as either low grade or high grade (ISUP system) and as either non-invasive or invasive (into lamina propria or muscularis propria).

Results: Of the 108 biopsy proven UCC persistence or recurrences, 29 were detected as positive by urinary cytology (sensitivity = 27%), 55 were non-diploid (sensitivity = 51%), and 57 were either cytology positive or non-diploid (sensitivity = 53%). The greater sensitivity for UCC detection by DNA ploidy status vs cytology was more significant for low grade ($p = 0.0068$) versus high ($p = 0.0104$) tumors and for non-invasive ($p = 0.0008$) versus invasive ($p = 0.034$) lesions. Of 53 bladder washings that were reported as atypical by cytology, 21 (40%) were non-diploid ("positive") on DNA ploidy histograms.

Conclusions: In this study, DNA ploidy analysis significantly out-performed conventional cytology for detection of persistence or recurrence of UCC, subsequently proven on bladder biopsy. DNA ploidy status particularly out-performed cytology in the detection of low grade or non-invasive UCCs. We conclude that, when the cytology is reported as atypical but not definitive, DNA ploidy status provides important additional information that can be used to guide follow-up studies and overall clinical management for patients with UCC.

329 Cytospin Is Superior to ThinPrep for the Detection of CSF Involvement by Hematolymphoid Malignancies

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Background: The cytopathologic examination of cerebrospinal fluid (CSF) is an important diagnostic test in the evaluation and management of patients with subarachnoid spread from both primary and metastatic neoplasms. However, limited literature exists that evaluates the utility and potential diagnostic pitfalls of the different modalities used to examine CSF. The purpose of the current study was to compare ThinPrep with cytospin in the evaluation of CSF specimens.

Design: A computerized search of the Stanford Department of Cytopathology files from 2/02-9/02 disclosed 447 CSF ThinPrep specimens, 204 of which had concurrent air-dried cytospin slides from the Clinical Laboratory Hematology Service. The ThinPrep slides were prepared with Papanicolaou stain and the air-dried cytospin slides with Wright Giemsa stain. The two sets of slides were compared and the number of discrepant diagnoses measured. Two types of discrepancies were recorded: (1) Major = two degrees of difference (negative vs. suspicious, negative vs. positive, atypical vs. positive) and (2) Minor = one degree of difference (negative vs. atypical, atypical vs. suspicious, suspicious vs. positive). The discrepant diagnoses were then categorized as sampling or interpretative errors.

Results: 19 total discrepant cases were identified, including 12 major and 7 minor discrepancies (Table 1). The major discrepancies predominantly involved the interpretation of lymphoma cases on ThinPrep slides (7/12): Large B-cell lymphoma (4) and leukemia (3). 5/7 discrepancies were due to interpretative errors while 2/7 were due to sampling. The discrepant cases on the cytospin slides consisted of lymphoma (2) and metastatic carcinoma (3). 2/5 discrepancies were due to interpretive errors, 2/5 to sampling and 1/5 was not available for review. Morphologic review revealed that hematolymphoid cells on ThinPrep slides appeared small and bland and the classic morphologic features, including cytoplasmic and chromatin detail, were lost. In contrast, the hematolymphoid cells were readily identified as malignant on the air-dried cytospin slides.

Conclusions: In evaluating CSF samples for involvement by hematolymphoid malignancies, air-dried cytospin slides are superior to ThinPrep. If ThinPrep only is used, it is important to be aware that malignant hematolymphoid cells can appear small and bland.

Table 1. Summary of Discrepant Diagnoses

	Major Discrepancy (12)		Minor Discrepancy (7)	
	Carcinoma	Lymphoma	Carcinoma	Lymphoma
Cytopathology	0	7	0	5
Hematology	3	2	0	2

330 Characteristics of Pap Smears with Squamous Intraepithelial Lesions Missed by the ThinPrep Imager System

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Background: The ThinPrep Imager System (Cytyc Corporation, Boxborough, MA) was FDA approved in 6/2003 as an automated device to assist cytologists in screening gynecologic pap smears. The device gained approval because it was shown to be at least as effective as manual screening for detection of both high grade and low grade squamous intraepithelial lesions (HGSIL and LGSIL). The system scans the slide and selects 22 fields of view (FOV) for review by the cytologist. The system preferentially selects small dark cells for display to detect HGSIL if present. If the 22 FOV show no abnormality the slide can be signed out as negative for intraepithelial lesion or malignancy (NILM). If an abnormality is found the entire slide must be manually screened. Our hospital adopted the Imager system on 11/1/2005.

Design: Our cytology laboratory QA records were searched to find all cases in which the 22 FOV selected by the imager contained no abnormality, but manual resceening detected an abnormality.

Results: From 11/1/05 to 8/31/06 13484 ThinPrep gyn cytology cases were accessioned into our cytology laboratory. 582 were deemed unable to be screened by the imager system. Of the remaining 12902 cases, 11526 were NILM by review of the 22 FOV. 2250 cases were manually rescreened (either because of mandated 10% negative rescreening or because of new personnel), and 4 cases were found to have abnormalities not detected in the initial review because abnormal cells were not present in the 22 FOV. 2 cases were atypical squamous cells (ASC) and 2 cases were LGSIL. Close review of these cases revealed clusters of polymorphonuclear leukocytes in 3 of the cases, and clusters of squamous metaplastic cells in 1 case which were preferentially selected by the system to appear in the 22 FOV. Followup of these cases showed that one LGSIL case had concomitant LGSIL on biopsy. The other LGSIL case had a previous conization showing CIN 1, and followup Pap was atypical squamous cells. One of the ASC cases had positive high risk HPV, and the other had repeat pap showing NILM with no HPV testing sent.

Conclusions: The ThinPrep imager preferentially detects small dark cells in order to present possible HGSIL to the reviewer. We postulate that clusters of inflammatory cells and/or metaplastic cells sufficiently mimic HGSIL to the instrument to cause these benign groups to be included in the 22 FOV, and excluding the selection of the larger cells with lower N/C ratios of ASC and LGSIL. Knowledge of this phenomenon may allow closer scrutiny of such cases to detect low grade lesions.

331 D2-40 as a Mesothelial Marker in Peritoneal and Pelvic Fluid Cytologic Specimens: Its Comparison with Other Mesothelial Markers

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Background: The distinction between mesothelial cells and metastatic carcinoma in cytologic specimens often poses a significant challenge. Immunocytochemistry is often used to aid in the diagnosis. Several mesothelial markers are in existence, including the recently discovered D2-40 monoclonal antibody, which is reported to be a useful

marker of mesothelial cells. The objective of this study is to determine the expression of D2-40 in peritoneal and pelvic fluid cytologic specimens and to compare the efficacy of 5 commercially available mesothelial markers in differentiating mesothelial cells from metastatic carcinoma in peritoneal and pelvic fluid.

Design: Forty formalin-fixed, paraffin-embedded cell blocks were retrieved from the archives and immunostained with monoclonal antibodies directed against calretinin (clone Z11-E3; prediluted; Ventana, Tucson, AZ), mesothelin (clone 5B2; 1:60 dilution; Novocastra, New Castle, UK), cytokeratin (CK) 5/6 (clone D5/16B4; 1:25 dilution; Invitrogen, Carlsbad, CA), Wilms tumor (WT) 1 (clone 6F-H2; 1:50 dilution; Dako, Carpinteria, CA) and D2-40 (clone D2-40; dilution 1:100; Dako, Carpinteria, CA). Sixteen specimens (12 peritoneal, 4 pelvic) were negative for malignancy. Metastatic disease was present in 22 cases (16 peritoneal, 6 pelvic) and originated from the ovary, uterus, liver, peritoneum, stomach, colon and pancreas. Two cases of peritoneal mesothelioma were included. Positive staining was defined as nuclear staining with calretinin and WT-1, cytoplasmic staining with CK5/6, membranous staining with D2-40 and membranous and/or cytoplasmic staining with mesothelin. Follow-up included review of corresponding histology and medical records.

Results: The differences in the staining pattern between metastatic carcinomas and mesothelial cells were statistically significant ($p<0.05$) for each marker. Table 1 summarizes the sensitivity, specificity, likelihood ratio and accuracy for each marker.

Conclusions: All markers distinguish mesothelial cells from metastatic carcinomas in peritoneal and pelvic fluid specimens. D2-40 and calretinin are both sensitive and specific mesothelial markers and are superior to CK5/6, WT-1 and mesothelin.

Efficacy of various antibodies as mesothelial marker

	Calretinin	WT-1	CK 5/6	Mesothelin	D2-40
Sensitivity	94%	72%	50%	100%	94%
Specificity	100%	82%	100%	50%	100%
Likelihood Ratio	46.8	12.4	17.7	16.6	46.8
Accuracy	98%	78%	78%	73%	98%

332 Sensitivity and Specificity of Tao Brush Cytology for the Detection of Endometrial Malignancy

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Background: Numerous studies in the last decades have evaluated the feasibility of cytologic endometrial sampling as a screening and diagnostic method for endometrial malignancy with wide ranges of sensitivity and specificity. The purpose of this study was to determine the sensitivity and specificity of Tao Brush cytology for the detection of endometrial malignancy using histopathologic findings from hysterectomy specimens as the gold standard.

Design: Brushings of the endometrial cavity were obtained with a Tao brush (Cook Ob/Gyn, Spencer, IN) from 65 total abdominal hysterectomy specimens immediately prior to frozen section evaluation. The sample was fixed in Preservcyt and processed for cytologic analysis using the Thin Prep technique. Cytologic diagnoses were classified as negative for malignancy, atypical, or positive for malignancy. Histopathologic findings were used as the gold standard for determining the sensitivity and specificity of cytology which were calculated in two ways: 1) using only positive cytologic diagnoses as evidence of malignancy, and 2) using both positive and atypical diagnoses as an indicator of possible neoplasia needing further triage.

Results: Histopathologic analysis revealed that 34 of the 65 patients had malignancy: 26 endometrioid adenocarcinomas, 3 carcinosarcomas, 3 clear cell adenocarcinomas and 2 serous adenocarcinomas. The remaining 28 cases were benign. The cytologic findings for three cases were considered non-diagnostic and these cases were excluded from the analysis. The cytologic diagnoses for the remaining 62 cases were: positive for malignancy (N=23), atypical (N=18), and benign (N=21). The sensitivity and specificity of cytology for the detection of endometrial malignancy are shown below.

Sensitivity and Specificity of Endometrial Cytology

	Positive only*	Positive and atypical*	p value
Sensitivity	21/34 (62%)	32/34 (94%)	0.001
Specificity	26/28 (93%)	19/28 (68%)	0.016

*Criteria for endometrial cytologic diagnosis to be considered evidence of malignancy.

Conclusions: This study indicates that endometrial cytology by the Tao brush is a sensitive method for detecting neoplasia if "positive" and "atypical" assessments are used as indicators for potential triage to more specific methods of diagnosis.

333 Positive Predictive Values of "Suspicious for Follicular Neoplasm" Versus "Suspicious for Hurthle Cell Neoplasm" on Cytology: A 6 Year Retrospective Review

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Background: Fine-needle aspiration (FNA) has become an acceptable and cost-effective procedure for rapid diagnosis and triage of thyroid nodules. Some studies indicate a higher rate of malignancy in thyroid nodules with cytologic diagnosis of suspicious for Hurthle cell neoplasm. The aim of our study was to compare the rate of malignancy diagnosis in resected specimens in patients with cytologic diagnosis of "suspicious for follicular neoplasm (FN) " vs. "suspicious for Hurthle cell neoplasm (HN) ".

Design: Cytopathology slides and reports of thyroid FNA's interpreted as "suspicious for thyroid neoplasm" in the archived files of our institution from 2000-2005 were reviewed. Cases with a cytology diagnosis of "suspicious for FN" or "suspicious for HN" with who had surgical follow-up were selected. We investigated one hundred sixty five cases including 115 cases with "suspicious for FN" and 50 cases with "suspicious for HN" on cytopathologic diagnosis. The cytopathologic diagnoses were correlated with the histologic findings along with other parameters.Positive predictive value (PPV) was calculated.

Results: The cytologic diagnosis in the "suspicious for FN" and "suspicious for HN" category with the histologic correlates is depicted in the following Table1:

Correlation of Histologic Diagnosis and Cytologic Diagnosis

CD\SD	AN	BN	FC	HC	PC	OC
Suspicious of FN (n=115)	24%	48%	13%	0	14%	1%
Suspicious of HN (n=50)	16%	44%	4%	14%	18%	4%

CD:Cytologic Diagnosis, SD:Surgical Diagnosis

The surgical pathologic diagnosis of 115 cases with "suspicious for FN" on cytologic diagnosis were 28 adenomatous nodules (AN), 55 benign neoplasms (BN), 15 follicular carcinomas(FC), 16 papillary carcinomas (PC) and 1 other carcinomas (OC). The surgical pathologic diagnosis of 50 cases with "suspicious for HN" on cytopathologic diagnosis were 8 AN, 22 BN, 2 FC, 7 HC, 9 PC and 2 OCs. The diagnosis of "suspicious for FN" on cytology showed a PPV of 27.8%. Whereas PPV in diagnosis of "suspicious for HN " on cytology was 40%.

Conclusions: Although it was not statistically significant, the PPV in "suspicious for HN" was higher than in "suspicious for FN" on cytologic diagnosis.

334 Cervical Dysplasia in NIL/M Pap Smears with HPV DNA Positive Testing

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Background: The utility of human papillomavirus (HPV) DNA testing as a screening tool for cervical cancer is not clear. An increasing number of clinicians at our institution request HPV DNA testing regardless of cytopathology diagnosis. Amongst out clinicians, NIL/M Pap smears with HPV positive tests are referred to colposcopy, a deviation from the recommendation of repeat cytology and HPV test in 6-12 months as proposed by the NCI-ASCCP Interim Guidance. This allows for retrospective analysis of negative pap smears with positive HPV tests and immediate colposcopic and subsequent colposcopic follow-up. The aim of this study was to retrospectively review colposcopy material from women with positive HPV testing and negative Pap smear cytology.

Design: We reviewed 49 NILM Pap smears with automatic HPV testing between May 2004 and December 2005 that had colposcopy follow-up after search of the laboratory information system database following local IRB approval. All histologic material collected after the NILM Pap smear was reviewed. The cases were examined with regard to history of dysplasia, presence of HPV, and detection of dysplasia on subsequent colposcopic exam. Pap smears from all high grade lesions were re-reviewed or results of 5-year retrospective analysis were noted.

Results: The LIS search identified 49 cases of negative Pap smears with 42 HPV positive cases and 7 HPV negative cases. The patients ranged in age from 17 to 63 years (mean = 34 years). Average time to follow-up was 14 weeks with follow-up extending to 2 years. Within the HPV positive group, 36% of women had a history of dysplasia. High grade lesions were identified in 38% of the HPV positive group within an average of 32 weeks. High grade dysplasia was found in 40% of women with a history of dysplasia and 37% of women with no history of dysplasia. No high grade lesions were identified in the HPV negative group. The incidence of HPV positive ASC-US reflexively tested in our laboratory did not change during the study period and the population remained stable.

Conclusions: A positive HPV test and a normal Pap smear reflect increased risk for either missed or small lesions not identified with cytology alone and the subsequent development of CIN 2/3 lesions or carcinoma. HPV testing may have an application in screening a high risk population regardless of cytologic diagnosis. Prospective multi-institutional trials and enhanced clinical algorithms for HPV triage are needed.

335 Pre-Operative Diagnostic Assessment of Pseudocysts of the Pancreas: A Multimodal Approach

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Background: It is imperative to distinguish pseudocysts (PCs) from cystic neoplasms of the pancreas, in particular mucinous cystic tumors. Little data concerning the cytomorphological spectrum of PCs of the pancreas is currently available. We document the clinical, cross sectional imaging findings, cyst fluid analysis and the cytologic spectrum of PCs.

Design: 36 histologically confirmed pseudocysts of the pancreas were evaluated. The clinical, cross sectional imaging findings (n=30) and cyst fluid data (n=17) were recorded. A cyst fluid CEA of > 192 ng/ml was considered elevated. 84% of aspirates were endoscopic ultrasound guided. Cytologic material was reviewed in all cases. We recorded the presence of histiocytes, neutrophils, pigmented debris, gastrointestinal (GI) contaminating epithelium, atypical epithelial cells, fat necrosis, and background mucin. Alcian blue and mucicarmine stains were available in 19 cases.

Results: The mean age was 36 years (range 22 to 76). Sixteen patients were males and 20 females. Thirty two (94%) of cases presented with chronic and/or acute pancreatitis. Cross sectional imaging favored a diagnosis of a pseudocyst in 24 (80%) cases. Five cysts (17%) were not further characterized, while a diagnosis of cystadenocarcinoma was suggested in one case. Cyst fluid CEA levels were elevated in 2 cases (17%). The median amylase level was 35392 U/L (range 2280 to 246002). Prospectively, on cytology, 32 cysts (89%) were classified as negative for malignancy, 3 as atypical, and one as suspicious for malignancy. Retrospective analysis revealed atypical epithelial cells in 3 cases. Pigmented extracellular debris was seen in 21 (42%) of cases. Fat necrosis was evident in 4 cases. Four (11%) cases showed GI contaminating epithelium. 24 cases (67%) showed macrophages, and 18 (50%) neutrophils. Four cases (11%) showed scant mucin on a Papanicolaou stain, while minimal amounts of extracellular Alcian blue/mucicarmine positive material was evident in 6 cases (33%).

Conclusions: A history of pancreatitis, corroborative cross sectional imaging data, a cyst fluid CEA of <192 ng/ml and amylase of >2000 U/L provides strong support for a diagnosis of PC. Although cytologically PCs are characterized by pigmented debris and lack of mucin, the cyst contents of a PC are often non-specific. Atypical epithelial cells occasionally cause diagnostic difficulties. Accordingly, correlation with imaging and cyst fluid analysis data would allow for a more specific cytologic diagnosis, and avoid misinterpretation of epithelial elements.

336 Papanicolaou Tests (PT) with Glandular Abnormalities: Impact of the 2001 Bethesda Classification

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Background: Besides changing the name AGUS to AGC to avoid confusion with ASC-US, Bethesda 2001 (B2001) classifies glandular cell abnormalities as atypical endocervical, endometrial, or glandular cells, NOS or favor neoplastic. Since the finding of AGC is clinically important due to a relatively high percentage of underlying significant squamous or glandular disease (10-39%), the qualifier of "favor reactive" was abandoned. A new category for AIS was also introduced. We have reviewed our experience with the diagnosis and follow-up of glandular abnormalities before and after the introduction of B2001 to detect any changes that were brought about by its implementation.

Design: We studied our cytopathology database to identify all reported cases of AGUS and AGC, three years before and after the introduction of B2001 at our institution, from 01/01/2000 to 12/31/2005. Information extracted from the records included age, type of cytologic preparation, hormonal status, microorganisms, associated SIL, interval to follow-up, type of biopsy and final biopsy results.

Results: A total of 387,278 PT were processed in our lab during the study interval, when our institution also gradually introduced liquid-based Papanicolaou tests (LBPT) (Surepath). The rate of reported glandular abnormalities pre B2001 (371/198,716, 0.19%) and post B2001 (380/188,562, 0.20%) remained unchanged. The biopsy rate was similar between the two periods: pre-B2001 it was 221/371 (59.6%)whereas post-B2001 it was 240/380 (63.2%) (p=1.00). Only 7 cases of AIS (1.8% of all glandular abnormalities) were reported:; 6 were biopsied and 4 showed AIS or endocervical adenocarcinoma. The rate of significant abnormalities (CIN2/3, AIS or adenocarcinoma) in follow-up biopsies of cases reported as AGUS (23.1%) and AGC (17.1%) was also similar (p=0.20). However, fewer CIN2/3 lesions were found in the follow-up of AGC (8/240, 3.3%) than in the follow-up of AGUS (19/221, 8.6%) ($p \leq 0.025$). There also was an increase in the percentage of glandular abnormalities reported as adenocarcinoma on PT 16/371 (4.3%) pre-B2001 versus 31/380 (8.2%) ($p \leq 0.05$). However, these two differences probably reflect the higher prediction for glandular abnormalities made possible by LBPT rather than the influence of B2001.

Conclusions: B2001 did not significantly change the rate of AGC diagnosis or the rate of significant abnormalities found in follow-up biopsies of cases diagnosed as AGC.

337 Does Human Papillomavirus (HPV) Genotyping Predict Biopsies with High Grade Dysplasia (CIN2/3) Following a Liquid-Based (Surepath) Pap Test Diagnosis of LSIL?

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Background: The major goal of cervical cancer screening by Papanicolaou tests (PT) is to identify and treat significant precursor lesions (CIN2/3) thus preventing their progression to cervical cancer. PT are diagnosed as LSIL in about 2.5% of all women screened and are the second only to ASC-US in frequency. However, based on ALTS data, no triage scheme has been recommended for LSIL to date, and the ASCCP guidelines recommend immediate colposcopy for women with this PT diagnosis. LSIL PT interpretations have been shown to be associated with underlying CIN2/3 lesions in 10-20% of follow-up biopsies. The aim of this study is to assess the value of HPV genotyping to predict underlying CIN2/3 in women with PT diagnoses of LSIL.

Design: The computerized records of our institution were searched for liquid-based PT diagnosed as LSIL from 04/01/2001 to 9/30/2005 on which HPV genotyping was performed. HPV identification was performed on residual Surepath PT samples by PCR using the MY09/11 primers and typing was performed through RFLP. HPV types were classified according to Munoz, et al, 2003, into high risk, probable high risk and low risk. The HPV results were then correlated to the follow-up biopsies obtained within 6 months of the index PT.

Results: 392 PT diagnosed as LSIL during this period had both HPV genotyping and follow-up biopsies available. Of these, 98 (25%) were HPV-negative, 243 (62%) showed 1 HPV type, and 51 (13%) showed two or more HPV types. hr-HPV was present in 37.8%, phr-HPV in 18.4%, while the remaining HPV types were either low-risk or untypeable. HPV16 was present in 68 cases (17.3%) overall. Of the cases with follow-up biopsies showing no CIN, CIN1 and CIN2/3+ HPV16 was present in 10%, 15% and 40% respectively. 67/392 (17%) of cases with PT diagnosis of LSIL had a subsequent biopsy diagnosis of CIN2/3. The presence of HPV16 was associated with CIN2/3 in 29% of cases , while all other high-risk HPV types were associated with a 7% likelihood of follow-up diagnosis of CIN2/3 ($p<0.001$).

Conclusions: Our data indicate that there may be a role for HPV genotyping in the management of LSIL, since the presence of HPV16 is associated with an increased risk of underlying CIN2/3, that may warrant a closer follow-up.

338 Fine Needle Aspiration Cytology of Primary and Metastatic Papillary Renal Cell Carcinoma: A Study of 45 Cases

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Background: Although papillary renal cell carcinoma (PRCC) has distinct histologic features, its cytologic features in fine needle aspiration (FNA) specimens are not well described. We retrospectively evaluated cytopathologic features and the utility of immunocytostaining for the FNA diagnosis of PRCC.

Design: By searching our institutional pathology database between January 1995 to July 2006, we identified 45 FNA cases (from 43 patients, 39 men and 4 women) that had slides available for review. Of the 45 samples, 24 were from kidney lesions that were histologically confirmed as PRCC and 21 were from metastatic tumors that were finally proven to be metastatic PRCC based on clinical history, morphologic comparison with matched primary PRCC and/or immunostaining.

Results: Tumor size ranged from 1 to 15 cm for kidney lesions and from 1 to 21 cm for metastatic lesions. FNA diagnoses rendered for the kidney lesions were PRCC (n=19), renal cell carcinoma, NOS (n=3), high grade carcinoma (n=1) and probably urothelial carcinoma (n=1); diagnoses for extra-kidney lesions were metastatic PRCC (n=16) and metastatic renal cell carcinoma, NOS (n=5). Cytologically, smears showed moderate to high cellularity in 44 of 45 samples (98%), papillary structures in 32 samples (71%), numerous single cells in 36 (80%), granular cytoplasm in 31 (69%), cytoplasmic vacuoles in 8 (18%), cytoplasmic hemosiderin in 4 (9%), prominent nucleoli in 17 (38%), and nuclear grooves in 26 (58%). In the background, foamy histiocytes were found in 21 cases (47%), and psammoma bodies in 4 cases (9%). Immunostaining had been conducted at the time of diagnosis for 9 primary and 4 metastatic tumors, mostly in cases in which papillary structure, cytoplasmic hemosiderin and foamy histocytes were not present. Tumor cells expressed vimentin in 11 of 11 cases, CD10 in 5 of 6, CK7 in 6 of 7 and P504S in 4 of 4 cases tested, but did not express CK20 or thrombomodulin in 5 of 5 cases tested.

Conclusions: The presence of papillary structure, cytoplasmic hemosiderin and foamy histocytes helps in reaching a diagnosis of PRCC, but the cytologic features often overlap with those of other subtypes of renal cell carcinoma. Clinical history and immunostaining are important to avoid misclassification in primary setting and to exclude tumor of other primary origin in metastatic setting.

339 Virtual Slide (Whole Slide Scan) Telecytology Using Surepath Papanicolaou Tests: A Feasibility Study

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Background: Papanicolaou test (PT) telecytology using still images has had only limited success, since the slide context from which the images were taken is missing. Whole slide scans offer the possibility of telecytology where the entire slide can be assessed by the remote pathologist. This study addressed the feasibility of remotely diagnosing Surepath Pap tests after they have been screened and dotted by a cytotechnologist.

Design: 20 Surepath slides were selected to reflect a variety of diagnoses and were reviewed by a senior cytotechnologist and a cytopathology fellow to independently confirm the original diagnosis. All cases of HSIL and above also had histologic correlates of CIN2/3+. The slides were scanned at 40x with the original dots placed by the cytotechnologists using the Aperio CS scanner and were stored on a dedicated server. Pathologists from 4 academic institutions were invited to access these slides through the internet using the Aperio ImageScope software or a Flash application and render their diagnoses. The case mix was unknown to the participants.

Results: 20 Surepath slides having 1-27 "dots" (average 9) were scanned resulting in files that after compression were 147-336 MB in size. The average time to scan a Surepath slide was 13 minutes. The reference diagnoses were NILM (n=12), LSIL (n= 2) and HSIL (n=6). Five board certified cytopathologists from 4 academic institutions participated. 12 of the 20 slides showed unanimous agreement of the 5 pathologists to the reference diagnosis and an additional case showed agreement within the category of the reference diagnoses (i.e ASC-US to LSIL; ASC-H to HSIL). The remaining 7 cases showed only minor disagreements (i.e ASC-US to NILM). Of the 6 cases of HSIL there was unanimous agreement with the reference diagnosis in 5 cases, while the sixth had one participant diagnosing LSIL while the others diagnosed HSIL.

Conclusions: Our results show that scanning the whole Surepath slide at 40x is feasible, is relatively fast and results in large files that can, however be easily viewed through the internet. The performance of the pathologists participating in this study was very good, since all HSIL cases were identified either by all 5 pathologists or by 4/5 pathologists.

340 Should "Low-Grade Squamous Intraepithelial Lesion, Cannot Exclude High-Grade Squamous Intraepithelial Lesion (LSIL-H)" Be a Distinct Cytologic Category?

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Background: The goal of cervical cancer screening is to identify and treat women with high-grade squamous intraepithelial lesions (HSIL). Cytologic interpretations for squamous epithelial abnormalities defined in the 2001 Bethesda System include atypical squamous cells, cannot exclude HSIL (ASC-H), low-grade squamous intraepithelial lesion (LSIL), and HSIL, each of which has a defined risk of associated cervical intraepithelial neoplasia 2 or more severe lesion (CIN2+) on histologic follow-up. Findings of unequivocal LSIL plus ASC-H (LSIL-H) on Papanicolaou (Pap) tests are currently not considered a separate cytologic category. Defining LSIL-H as a distinct cytologic category may be justified if the histologic outcomes and prevalence of high-risk (HR) HPV differ significantly from established categories.

Design: The Johns Hopkins Hospital Pathology database was searched for liquid-based Pap tests with cytologic interpretations of LSIL, LSIL-H, ASC-H, and HSIL during the period of 1/1/03-12/31/03. For each patient, the most severe lesion on histologic follow-up for up to 2 years was recorded. For LSIL-H and ASC-H, results of concurrent Hybrid Capture 2 (Digene) HR HPV DNA testing were noted. Statistical analysis was performed using the Fisher exact test.

Results: Histologic follow-up was available for 426/690 (62%) LSIL, 81/113 (72%) LSIL-H, 86/122 (70%) ASC-H, and 110/131 (84%) HSIL interpretations. The risk of histologic CIN2+ associated with LSIL-H (39.5%) was intermediate between LSIL (10.8%, $p<0.0001$) and HSIL (65.5%, p=0.0006), and not statistically different from ASC-H (26.7%, p=0.1118). LSIL-H was more frequently associated with a

definitive histologic diagnosis of any CIN (CIN1+) compared to ASC-H (61.7% vs 37.2%; p=0.0025). Among patients who had histologic follow-up and HPV testing, the prevalence of HPV was significantly greater in LSIL-H than in ASC-H (100% vs 59%, p=0.0019).
Conclusions: The risk of underlying histologic CIN2+ associated with LSIL-H is similar to ASC-H, significantly greater than unqualified LSIL, and significantly less than HSIL. Patients with LSIL-H and ASC-H differ in their risk of harboring any CIN. Moreover, the difference in prevalence of HPV among patients with LSIL-H and ASC-H suggests that while HPV testing is not useful in the management of LSIL-H, it may have a potential role in the management of ASC-H. Thus, LSIL-H should be considered a distinct cytologic category that warrants close clinical follow-up of patients to exclude underlying CIN2+.

341 Atypical Squamous Cells of Undetermined Significance (ASCUS): A Valid Diagnostic Category in Anal Cytology?

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Background: Anal exfoliative cytology (AEC) is a useful screening method in high risk individuals for detecting anal squamous intraepithelial lesions (SIL). Although not endorsed officially for AEC, most practicing cytopathologists apply The Bethesda System of cervical cytology in reporting anal smears. One common and diagnostically frustrating category often employed is ASCUS. We sought to determine the significance of this diagnosis by comparing it to histologic and cytologic follow-up in a single large series.
Design: All AEC cases with a diagnosis of ASCUS were identified from the cytopathology archives of a large teaching hospital. Only cases with adequate follow-up (histology and/or repeat cytology for a minimum of 2 years) were included in the final analysis. Pertinent clinical information including HIV status was noted. The risk of progression to anal intraepithelial neoplasia 2 (AIN 2) or worse was calculated.
Results: Of the 109 cases identified, 83 (M:F ratio 11:1, age range 26-65, mean 42) had adequate follow-up. Of these, 24/83 (29%) had negative follow-up, 23/83 (27%) had AIN 1, 25/83 (31%) had AIN 2 or worse and 11/83 (13%) continued to have "atypical" follow-up (ASCUS, or biopsies with atypia) by 2 years of the sentinel ASCUS smear. Follow-up on HIV negative individuals (n=13) revealed 9/13 (69.2%) had benign follow-up, 3/13 (23.1%) AIN 1 and 1/13 (7.7%) had high grade lesions by two years. During the same period of time, analysis of all AECs revealed 509 total cases; 109 (21.4%) were ASCUS, 189 (37.1%) were AIN 1, 25 (4.9%) were AIN 2-3 and 186 had (36.5%) no abnormality.
Conclusions: 1) The incidence of SIL in AEC is high (42%) with 4.9% of all cases showing AIN 2 or worse. 2) ASCUS in AEC is a valid diagnostic category, having a high rate of progression to AIN 2 or 3. 3) ASCUS in HIV negative individuals is far less ominous than HIV positive individuals with much lower rate of progression to high grade SIL. 4) ASCUS in AEC warrants close clinical follow-up/management due to a higher incidence of SILs than the same diagnosis in cervical cytology. 5) In HIV negative individuals ASCUS on AEC progresses to high grade lesion less frequently than in HIV positive individuals. Therefore less rigorous follow-up could be implemented such as repeat pap or triage with HPV molecular test as opposed to anoscopy for HIV positive individuals.

342 Utility of p16INK4A Immunohistochemistry in Differentiating Cystic Squamous Cell Carcinoma from Branchial Cleft Cyst in Fine Needle Aspiration Biopsies of the Head and Neck

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Background: Cytologic distinction of metastatic cystic squamous cell carcinoma (SCC) from benign squamous cell lesions, such as branchial cleft cysts (BCC), can be very challenging. Recently, p16INK4A has been shown to be overexpressed in squamous cell carcinomas, particularly of the oropharynx. The purpose of this study is to evaluate the utility of p16INK4A immunohistochemistry in distinguishing cystic well-differentiated squamous cell carcinoma from branchial cleft cysts in fine needle aspiration specimens.
Design: The study set consisted of twenty-six cases of surgically excised cystic lesions of the neck comprising 16 branchial cleft cysts and 10 well-differentiated squamous cell carcinomas. A test set of paraffin-embedded cell block material from fine needle aspiration biopsies of three branchial cleft cysts and four cystic squamous cell carcinomas were also evaluated. p16INK4A immunohistochemical staining (Dako, Carpinteria, CA) was scored as focal or diffuse and weak or strong.
Results: Overall, 6 of 10 cystic SCC in the study set exhibited diffuse, strong reactivity for p16INK4A with all positive cases representing a metastasis from either the base of tongue or tonsil. Staining was limited to the malignant nucleated squamous cells with no expression identified in the keratinous debris within the cyst cavity. Three of the four cystic SCC in the test set exhibited diffuse, strong positivity. All cases of metastatic cystic SCC from non-oropharyngeal primary sites were negative. Overall, 7 of 16 BCC (44%) in the study set exhibited focal, strong positive staining for p16INK4A. In all BCC cases, expression was limited to the superficial cyst-lining squamous cells with no staining seen in the keratinous debris within the cyst cavity. However, two cases showed diffuse strong staining within the glandular epithelium. All three test set cases of BCC were p16INK4A negative.
Conclusions: These preliminary results suggest that p16INK4A immunostaining can be a useful adjunct in differentiating benign and malignant squamous lesions in cell-block material. Diffuse strong p16INK4A reactivity in fragments of epithelial cells is required to support a diagnosis of malignancy. The absence of staining or focal strong staining would be considered equivocal since these patterns of staining can be seen with BCC, as well as cystic SCC.

343 Fine-Needle Aspiration Cytology of the Thyroid Follicular Lesion: Correlation with Histologic Findings

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Background: Fine-Needle Aspiration (FNA) is often the first step in management of a thyroid nodule. Although FNA has been used with success in the diagnosis of papillary, medullary, and anaplastic carcinomas, it is difficult to assess its value of follicular lesions. The main problem is the limited cytologic features in distinction between follicular malignancy and benign lesions, such as follicular adenoma or adenomatous goiter.
Design: A computer search of all cases of thyroid follicular lesions diagnosed by FNA at our institute from January 2001 to December 2005 was performed. The cytology and pathology reports of the patients were reviewed and correlated with follow up histologic findings.
Results: From January 2001 to December 2005, 1306 thyroid FNAs were performed. 261 cases (20%) were diagnosed as follicular lesion. The distribution by sex was 102 female and 19 male patients with the mean age of 48 years (median, 50; range, 21 to 78). Of the 261 patients with follicular lesion, 121 (46%) underwent thyrodectomy. The patients were divided into two groups on the basis of the final histologic diagnosis. Eighty-five patients (70%) had a final diagnosis of benign lesions and thirty-six (30%) had a malignant diagnosis. However, six of these malignant cases were incidental papillary carcinoma that did not involve the primary nodule. Therefore, the index nodule was benign in 91 patients (75%) including 6 patients with occult papillary carcinoma and malignant in 30 patients (25%). The detailed pathologic diagnosis was summarized in Table 1.

Table 1. Histologic diagnosis in 121 patients with follicular lesions

Histopathology	No. of cases	%
Colloid nodule	52	43
Follicular adenoma	22	18
Thyroiditis	8	7
Other benign	3	2
Papillary ca	9	7
Follicular variant of papillary ca	6	5
Occult papillary ca	6	5
follicular ca	9	7
Hurthle cell ca	2	2
Other malignancy	4	3

Conclusions: The diagnosis of follicular lesion carries a high risk of malignancy (25%) in our study. The classic cytologic features (nuclear grooves and intranuclear inclusions) for papillary carcinoma were absent in all 15 papillary carcinomas. No specific cytologic features in distinction follicular adenoma from follicular carcinoma were noted. No correlation was found between the malignancy and the patients' age, sex, and the nodular size. We believe that all follicular lesions should be excised to exclude the malignancy.

344 Reasons and Clinical Implications of Indeterminate Diagnosis in Fine Needle Aspiration (FNA) of the Pancreas

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Background: Indeterminate diagnoses (i.e., atypical cells or suspicious for carcinoma/neoplasm) rendered on FNA samples of the pancreas can cause dilemmas in clinical management. The purpose of this study was to determine the underlying reasons and clinical implications for these diagnoses.
Design: Sixty-five pancreatic FNA (58 via EUS and 7 via CT) samples from 56 patients (39 men and 17 women) with indeterminate diagnoses between 01/2000 and 07/2006 were retrospectively reviewed. The patient's mean age was 65 years (range, 30-92 years). Slides were available from review for 51 samples. A final diagnosis was determined based on the combined information of follow-up pathologic diagnoses (repeat FNA or subsequent resection) and clinical and radiographic findings.
Results: Thirty-eight FNA samples were initially diagnosed as atypical and 27 as suspicious. Radiologically, lesions ranged in size from indistinct to 10 cm (mean, 3 cm); 21 lesions (25 FNA samples) were cystic. Pathologic confirmation via repeat FNA and/or histologic follow-up was available for 42 (75%) lesions: 27 adenocarcinomas, 3 microcystic adenomas, 2 mucinous neoplasms without dysplasia, 1 mucinous neoplasm with high-grade dysplasia, 2 islet cell tumors, 2 intraductal papillary mucinous neoplasms, 1 neuroendocrine carcinoma, 1 adenomyoma, 2 chronic pancreatitis, and 1 autoimmune pancreatitis. Eight (14%) lesions had only clinical and/or radiologic follow-up: benign/indolent clinical course in 5 and malignant progression in 3. The remaining 6 (11%) patients were lost to follow-up or died of unknown causes. Overall, among the 50 tumors with follow-up information, 32 (64%) were malignant tumors, 10 (20%) were benign or borderline tumors and 8 (16%) showed benign pathologic follow-up and/or benign clinical course. The factors that contributed to an indeterminate diagnosis included scant cellularity, minimal atypia, cohesive flat cellular sheets, tumor cells admixed with abundant benign ductal cells or normal GI epithelium, inflammation-related cellular atypia and poor cellular preservation.
Conclusions: Most lesions with an indeterminate diagnosis on pancreatic FNA samples were malignant. A cystic nature and atypical cells that were quantitatively and/or qualitatively fallen short of diagnostic criteria contributed most to an indeterminate diagnosis. A repeat sampling for definitive diagnosis was often required. For those without subsequent pathologic confirmation, correlation with clinical and/or radiographic findings was important for appropriate clinical management.

345 Diagnostic Efficacy of Fine Needle Aspiration Cytology To Determine the Origin of Metastasis of Unknown Primary

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Background: Metastasis of unknown primary origin (MUP) constitutes 5 to 10% of all non-cutaneous malignancies. Fine needle aspiration cytology (FNA) is highly accurate in the diagnosis of metastatic disease. Moreover, cytopathologists are increasingly asked to determine the primary site of MUP. We evaluated the diagnostic accuracy of FNA including the value of immunocytochemistry (ICC) to determine the primary site of MUP.

Design: We retrieved the cytology report for all the FNAs performed at our institution from January 2000 to May 2006. We included cases that had a diagnosis of metastatic cancer (or possible metastatic cancer). We excluded cases with previous pathologic history of the same malignancy in the computer system, and cases with clinical history of primary site or information of possible primary (i.e. a mass in another anatomic site) provided in the requisition. We compared the FNA diagnosis with follow-up pathology and/or clinical information available in the electronic medical record or the cancer center registry.

Results: We evaluated 153 FNAs from 74 females and 79 male patients, with an age range of 20 to 88y (mean 63.7y). In 55/153 (36%) of the cases the FNA suggested at least one primary site. A primary site was subsequently confirmed by surgical pathology and/or clinical follow-up in 108/153 (71%) of these cases. Among the 108 with primary site determined by follow-up, the FNA correctly suggested the diagnosis in 31/108 (28.7%), was incorrect in 3/108 (2.8%), and didn't suggest a specific primary site in 74/108 (68.5%) of the cases. However, when ICC was performed in 80/153 (52%) of the cases, the percentage of cases in which the primary site was suggested was significantly higher (55%) than cases without ICC (15%). If we exclude the squamous cell carcinomas (SCC) from the analysis, there were 86 of 124 cases with follow-up, and the FNA correctly suggested the diagnosis in 30/86 (34.9%). The most common primary sites for MUP in our FNA series were unknown (31%), lung (24%), oral cavity/larynx/pharynx (12%), breast (6%), kidney (3%), and pancreas (3%).

Conclusions: FNA can correctly identify the primary site of MUP in slightly less than a third of cases in general, and more than a third of the non-SCC cases. When ICC is also performed on the FNA the primary site of MUP can be identified in more than half of the cases. These findings suggest that when FNA of MUP case is encountered, a cell block and or/ core biopsy should be obtained for ancillary ICC studies.

346 Fine Needle Aspiration (FNA) Cytology of Metastatic Spindle Cell Melanoma: A Study of 81 Cases

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Background: Spindle cell melanoma is an unusual morphologic variant of melanoma. In the metastatic setting, a diagnostic dilemma on FNA specimens may occur in regard to the primary origin and the differential diagnosis. In this study, we examined our experience with this entity and retrospectively reviewed its cytologic features.

Design: Eighty-one FNA cases from 70 patients with a cytologic diagnosis of spindle cell melanoma or melanoma with spindle cell features rendered between January 2000 and July 2006 were available for review. The aspirated sites included soft tissue (35), lymph node (22), lung (12), liver (5), pancreas (4), breast (1), parotid gland (1), and bone (1). Cytologic features and report of each case were reviewed.

Results: Cytologically, smears were moderately or highly cellular in 63 cases (78%), Specimens were composed of predominantly spindle cells in 62 cases (77%) and of mixed spindle and epithelioid cells in 19 cases (23%). Tumor cells were predominantly cohesive in 20 cases (25%), predominantly dyshesive in 19 cases (23%), and mixed cohesive and dyshesive in 42 cases (52%). Mild pleomorphism with bland nuclear features was found in 29 cases (36%) and moderate to marked pleomorphism was noted in 52 (64%). Mitosis and necrosis were rarely identified. Notably, cytoplasmic melanin pigment was found in only 12 cases (15%) and the features frequently noted in epithelioid melanoma such as binucleation and/or multinucleation, intranuclear pseudoinclusions, and macronucleoli, were readily found in 18 cases (22%), 29 cases (36%) and 17 cases (21%), respectively. Of interest, longitudinal nuclear grooves were found in 27 cases (33%). On the basis of cytologic reports, the diagnosis was facilitated by morphologic comparison with previous or concurrent pathologic materials (29 cases [36%]) and/or immunoperoxidase studies (32 cases [40%]).

Conclusions: Cytologic features of spindle cell melanoma can vary, from those resembling benign fibroblastic cells to those indistinguishable from high-grade sarcomatous neoplasm. Features frequently seen in epithelioid melanoma are noted infrequently in spindle cell melanoma. Therefore, clinical correlation, morphologic comparison and immunostaining are often needed to avoid misinterpretation.

347 The Cytomorphology of Low-Grade Endometrial Stromal Sarcoma: A Reveiw of 21 Cases

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Background: The cytomorphologic features of endometrial stromal sarcoma (ESS) have been incompletely described in a handful of reports. This study is a retrospective review of the cytologic findings of primary and metastatic ESS from a series of 21 cases including both gynecologic and non-gynecologic preparations.

Design: A fifteen year retrospective computerized search of our cytopathology files was reviewed for all cases of ESS. There were a total of 12 gynecologic and 9 non-gynecologic specimens, all from histologically proven low-grade ESS. The gynecologic preparations included 10 vaginal preparations (2 Thin Prep and 8 conventional smears) and 2 cervical preparations (Thin Prep). The 9 non-gynecologic specimens included fine needle aspirations of 3 soft tissue masses, 2 retroperitoneal masses and 3 pelvic masses and 1 diaphragmatic washing. The following cytologic features were assessed: overall neoplastic cellularity, cellular architecture, supporting stroma, nuclear and cytoplasmic features, and background material.

Results: Seventeen of 21 cases showed moderate to marked cellularity and a combination of single cells and clusters of cells. Three cases showed predominantly single cells with rare clusters and only one showed predominantly clusters with few single cells. More than half of the cases showed interspersed blood vessels between clusters of stromal cells and two cases had both interspersed blood vessels and hyaline matrix supporting the stromal cells. Six cases did not show any supporting matrix. The neoplastic cells were predominantly spindle-shaped with occasional epithelioid cells, and the cytoplasm ranged from scant to moderate. Few comet-shaped cells were identified. The nuclear contours were smooth, and the nuclei were predominantly round to ovoid with minimal anisonucleosis and anisocytosis. Fine chromatin was noted in 19 of 21 cases. Mitotic figures were seen only in 2 of 21 cases. Prominent nucleoli were identified in 3 of 21 cases. Multinucleated giant cells were seen in 3 of 21 cases. Background naked nuclei were common.

Conclusions: The most common cytologic features seen in low-grade ESS include the following: the presence of a combination of single cells and clusters of cells with interspersed blood vessels, predominantly spindle cells with fine chromatin and scant to moderate amount of cytoplasm with minimal anisonucleosis and anisocytosis. Metachromatic stroma with interspersed vessels, if present, is extremely helpful, however it is not a common finding.

348 Impact of Urine Cytology Errors on Patient Care

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Background: Although urine cytology is a mainstay for the diagnosis of urothelial cancer, there has been little study of the frequency, causes, and outcomes of urine cytology error.

Design: We obtained histologic follow-up in 362 voided (6.2%), 125 lower tract instrumented (19.5%), and 25 upper tract (34.2%) urinary cytologic specimens from 1 institution over a 2-year timeframe to determine diagnostic accuracy. A total of 4102 patients had a urine cytology during this timeframe. We reviewed the slides from specimens in which there was a diagnostic disagreement between the cytologic and histologic diagnoses and adjudicated the cause of discrepancy as sampling or interpretation failure. Clinical follow-up was obtained from the medical records in patients with discrepant diagnoses and outcomes were classified as no harm, near miss, and harm, and harm was further subdivided into minimal, mild, moderate and severe.

Results: Cytologic-histologic discrepancies were observed in 209 (40.8%) of cases with histologic follow-up, and the cause of discrepancy was interpretation and sampling in 34.9% and 63.2%, respectively. Of all discrepancies, 101 (48.3%) resulted in minimal or mild harm, consisting mainly of repeat invasive or non-invasive testing and diagnostic delays. Severe harm never was observed, as no patient developed invasive cancer as a result of a false negative urine cytologic diagnosis. Causes of error included poor specimen quality, diagnostic undercalls, and variable use of malignant and non-definitive diagnostic categories ($P < 0.001$). Only one of 7 pathologists was able to diagnose low grade cancers using urine cytology. For voided specimens, more cancers were detected by an atypical diagnosis (31% of all cancers) than by a malignant diagnosis (19% of all cancers). Considering an atypical diagnosis as predictive of a benign or neoplastic lesion markedly shifted diagnostic sensitivity for voided specimens from 49.2% to 74.6% for high grade and 9.7% to 50.5% for low grade cancer.

Conclusions: These findings indicate that cancer screening protocols with urine cytology are exquisitely accurate in not missing cancer but that potentially reducible errors result in unnecessary testing and diagnostic delays. Improved diagnostic standardization across pathologists and improving specimen processing techniques could effectively low errors.

349 Effectiveness of Cervical Cancer Screening Using Correlation Analysis

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Background: Although the limitations of cervical cancer screening are well known and the estimated sensitivity of a single Pap test is approximately 60%, the impact of cervical cancer screening correlation discrepancies is less understood.

Design: Four hospital labs measured the frequency of Pap test-histologic correlation discrepancy over a 6-year period. We calculated the discrepancy proportion using a denominator of total Pap tests. The labs standardized the correlation process in the mid-point of the study. Standardization was based on a 6-month look back between the histologic and Pap test specimens and discrepancies were defined as greater than a 2-step difference in the Pap test and histologic diagnoses. We adjudicated the cause of discrepancy as sampling, if tumor cells were not seen on either specimen, or interpretation, if diagnostic cells were seen on a slide on review. Outcomes were obtained by chart review and classified into the categories of no harm, near miss and harm. The category of harm was classified into minimal (e.g., delay in diagnosis), mild (e.g., repeat non-invasive test), moderate and severe.

Results: A total of 6,260 cytologic-histologic discrepancies were found 1,657,528 Pap tests (1 in every 265) across the 4 institutions. A missed squamous cancer occurred in 1 of every 166,000 Pap tests, although this discrepancy was adjudicated as secondary to interpretation error in only 1 of every 552,000 Pap tests. Approximately 40% of discrepancies resulted in harm, mainly classified as minimal or mild harm (delays in

diagnosis and repeat testing). Institutional discrepancy frequency varied from 0.14% to 0.62% of all Pap tests and reflected differences in correlation processes and/or biases in reporting. Histologic misinterpretation was the cause of 12% of all discrepancies.
Conclusions: Longitudinal, multi-institutional data show that cervical cancer screening using Pap testing is remarkably effective in preventing cervical cancer, as only 1 in 550,000 women had a missed squamous cancer. However, the problem with the current cervical cancer screening system is the large number of "low-impact" errors from over and under diagnosis resulting in over treatment.

350 Pancreatic Intraductal Papillary Mucinous Tumor (IPMT): A Cytomorphologic Study with Histopathological Correlation

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Background: IPMT is an increasingly recognized but rare entity, and its cytological features on fine needle aspiration cytology (FNAC) have not been well-established. We undertook this study to characterize the cytological features of this tumor in six cases with histological confirmation.
Design: The pathology files were searched for cytology and surgical cases diagnosed as IPMT over a 5 year period. Six cases with available cytology and histology slides were selected. The cytology slides were blindly evaluated for the following criteria: cellularity, architectural patterns, cell shape, nuclear morphology, mitoses, nuclear/cytoplasmic (N/C) ratio, intracellular mucin (ICM), extracellular mucin (ECM), background histiocytes, necrosis and inflammation. Cytological features were correlated with available histological, radiological, and clinical data.
Results: Patients ranged in age from 68 to 82 years (2 females, 4 males). Mean tumor size was 2.5 cm (range 1.2 – 4 cm). Four lesions were in the head, 1 in the body, and 1 in the tail of pancreas. Histologically, 5 cases harbored an in-situ or invasive adenocarcinoma (ACA), and the sixth was of borderline malignant potential. Moderately to highly cellular smears (MHCS) with large discohesive sheets of cells (LDSC) with anisonucleosis, moderate pleomorphism, irregular nuclear spacing and membranes (INSM), irregular chromatin, prominent nucleoli, and necrosis were seen in all 5 malignant cases. Tight, three-dimensional cell clusters (TTDCC) and papillary fragments of low-columnar and cuboidal cells with ICM, moderate amount of cytoplasm, hyperchromasia, nuclear overlapping, increased N/C ratio and background histiocytes were observed at least focally in all cases. Mitoses were seen in 4 of 5 malignant cases. A moderate to high amount of thick, viscous ECM was present in 83% of cases. Inflammation was seen in 1 malignant case.
Conclusions: 1) FNAC features of IPMT include TTDCC and papillary fragments of low columnar and cuboidal cells with ICM, moderate amount of cytoplasm, hyperchromasia, nuclear overlapping, and increased N/C ratio in a background of histiocytes and ECM. 2) Although thick, viscous ECM is moderately or extensively present in a majority of cases and should raise suspicion of IPMT on FNAC, its scant presence in a minority of cases may lead to diagnostic difficulty. 3) Malignant IPMTs additionally may show MHCS, necrosis, and LDSC with the classical cytological features of pancreatic ACA such as anisonucleosis, INMS, and irregular chromatin.

351 What Is the Accuracy of the Cytologic and Histologic Interpretation in Pap Smear and Cervical Biopsies? A Comparative Study Including HPV In-Situ Hybridization (ISH)

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Background: It is now accepted that high-risk human papilloma virus (HPV) is the major etiologic agent in the development of cervical cancer. In this study we confirmed the accuracy of cytological and histological interpretation with the presence of HPV in cytological preparation and histological material by in-situ hybridization (ISH) method (Ventana Inform HPV, Tucson, Arizona, USA).
Design: 203 consecutive cytology cases were collected and processed by Thin prep (Cytyc Corporation, Boxborough, Massachusetts, USA) and subsequently tested by HPV ISH. 154 (75.8%) cases underwent follow- up colposcopic biopsy within 6 months of original Pap smear, of which 127 biopsies were tested for HPV by ISH. Four pathologists and 2 cytotechnologists reviewed all test results.
Results: The results of HPV ISH on cytology specimens are as follows: 9/35 (25.7%) ASCUS, 15/17 (88%) HGSIL, 28/47 (60%) LGSIL and 6/28 (21.4%)NLM were positive by HPV ISH. Of a total of 127 follow-up biopsies, 96 (75.5%) were positive for cervical intraepithelial neoplasia with 66 (51.9 %) LGSIL and 30 (25.9%) HGSIL. On histology 26 (40%) cases of LGSIL, 21(70%) cases of HGSIL were positive by HPV ISH. Five of the HPV ISH negative HGSIL cases were positive by PCR. 31 biopsy-proven negative cases were negative by ISH.
Conclusions: Increased positivity in high grade cytological and histological diagnoses (where overcalls are low) provides us a preview that HPV ISH testing may be useful in reducing overcalls in LGSIL; however, determining the clinical sensitivity and specificity of such testing requires more studies and additional data.

352 Patients Diagnosed with ASC-US Followed by a Negative Reflex Test for High-Risk HPV Types: Comparative Longitudinal Cytologic Follow-Up in Routine Practice

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Background: The 2001 consensus guidelines essentially equated the follow-up management of patients with a cytologic diagnosis of "Negative for Intraepithelial Lesion or Malignancy" (NILM) with those diagnosed with "Atypical Squamous Cells of Undetermined Significance" (ASC-US) that was followed by a negative reflex test for high-risk (HR) HPV types: follow-up cytology in 12 months. These guidelines are based on the ALTS trial which utilized a histologic CIN3/cancer endpoint. Since the typical follow-up of these patients is cytologic, we sought to determine whether in routinely diagnosed cases, the full spectrum of follow-up cytologic findings are indeed identical in these 2 groups.
Design: Clinical and pathologic data of consecutive patients diagnosed with ASC-US during a 6-week period [n=587], in which reflex HPV testing was performed [n=497] and in which HR HPV types (Digene Hybrid Capture 2 assay) were ***not*** detected [n=300], were reviewed (study group). A randomly selected control group of 300 patients diagnosed with NILM during the same period were similarly reviewed.
Results: The study and control groups are summarized and compared below:
Conclusions: Some significant differences exist between these 2 groups. Patients in the ASC-US/negative HR HPV group are significantly more likely to have an abnormal follow-up smear than their counterparts with a NILM diagnosis; this is attributable to the more frequent repeat diagnoses of ASC-US in the former group.Our findings also suggest that these patients currently receive more intensive follow-up than patients diagnosed with NILM, which may be justified. However, the extraordinary rarity of the the most clinically significant diagnoses of HSIL and carcinoma in this setting can be confirmed, as no such cases were identified during the follow-up of both groups.

	Study Group	Control group	p value (Fisher's Exact Test)
Number of patients	300	300	N/A
Number with follow-up	226	190	N/A
Average follow-up (months)	26.03	25.9	N/A
Total # of follow-up smears	555	356	N/A
Average # of follow-up smears per patient	2.45	1.88	N/A
Any cytologic abnormality	138	27	<0.0001
NILM	417/555 (75.1%)	330/356 (92.4%)	<0.0001
ASC-US	114/555 (20.5%)	18/356 (5.1%)	<0.0001
LSIL	21/555 (3.8%)	9/356 (2.5%)	0.35
ASC-H	3/555 (0.5%)	0/356 (0%)	0.29
HSIL	0/555 (0%)	0/356 (0%)	N/A
CARCINOMA	0/555 (0%)	0/356 (0%)	N/A

353 Prediction of Invasive Disease on Conventional and ThinPrep Cervical Smears; an Analysis of Multiple Morphologic Features

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Background: To identify reliable morphologic criteria of invasion in both conventional and ThinPrep cervical smears.
Design: We analysed 7 well described morphologic features ie diathesis, nucleoli, tadpole cells, hyperchromatic dense black nuclei, pleomorphism, keratinisation and presence of microbiopsies in 11 cases of CIN3 and 11 cases of biopsy proven invasive squamous cell carcinomas in conventional preparations (CP) and 11 cases of CIN3 and 11 cases of biopsy proven invasive squamous carcinomas in ThinPrep (TP) preparations, while blinded to outcome. Each feature was statistically evaluated for both CP and TP by Fishers exact tests.
Results: Despite small sample size a trend towards significance was seen for presence of tumour diathesis (p=0.054) in CP's. No other feature approached significance. On TP's keratinisation of tumour cells approached significance (p=0.063) as a predictor of invasion. No other feature including diathesis approached significance on TP's.
Conclusions: These findings suggest that prediction of invasiveness on a cervical smear is difficult and that while tumour diathesis may be useful in conventional preparations, other features are less so. This is at variance with TP's where keratinisation of lesional cells seems to be the only reliable feature.

354 Intraoperative Thyroid Evaluation: Can Touch Imprints Improve Appropriate Surgical Management When Used in Conjunction with Frozen Sectioning?

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Background: While fine needle aspiration (FNA) is currently the standard diagnostic tool for thyroid lesions, many indeterminate cases after FNA are managed with intraoperative frozen sections (FS). The value of FS has been controversial, but it is still widely used to determine surgical management. We evaluated the role of intraoperative touch preparation (TP) in guiding surgical decision-making when used in conjunction with FS.
Design: A retrospective search for all thyroidectomy specimens with intraoperative evaluation was performed. 95 cases were identified that included both intraoperative cytology and histology. TP and FS were individually randomized and placed into separate boxes with the patient histories (age, gender, size and location of nodule). Two pathologists reviewed the material independently and rendered diagnoses for TP and FS separately. TP and FS diagnosis were correlated with the final diagnosis and it was determined whether or not the intraoperative diagnosis would have led to appropriate surgical management.
Results: 66 lesions were correctly identified as benign with no false positives (specificity 100%). Of the 29 malignant cases, sensitivity was 45% for FS alone (13 of 29 cases) and 55% for FS plus TP (16 of 29 cases). Overall, FS alone would have led to appropriate surgical management in 83% (79/95), which increases to 86% (82/95) when FS and TP are used together. 13 cases could not be identified as malignant on intraoperative evaluation: 3 follicular carcinomas showed capsular/vascular invasion on extensive permanent sampling and the remaining 10 cases were PTC, including 5 FVPTC. Two of the 10 PTC lesions were not sampled on frozen section and 3 were limited by poor quality of FS and TP. The remaining 5 cases, of which 3 are FVPTC, were difficult to interpret.
Conclusions: TP when used in conjunction with FS can slightly improve intraoperative diagnostic accuracy (sensitivity for malignancy 45% vs. 55%). The relatively small added benefit of intraoperative TP is likely due to pre-selection of difficult cases for intraoperative evaluation, i.e. cases that are indeterminate by FNA and which represent a disproportionate number of FVPTC (11/20).

355 Detection of *C-MYC* Rearrangement on Destained Fine Needle Aspirate Smears by Fluorescence In-Situ Hybridization

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Background: Burkitt lymphoma is a highly aggressive B-cell lymphoma that requires rapid diagnosis and treatment. Fine needle aspiration (FNA) provides a rapid, cost-effective tool in the diagnosis of Burkitt lymphoma. However, definitive diagnosis by FNA often requires confirmation of *C-MYC* rearrangement. This study evaluated *C-MYC* rearrangement in Burkitt lymphoma using fluorescence in situ hybridization (FISH) in destained FNA smears.

Design: FISH was performed on Romanowsky stained, archival FNA biopsies from 9 patients with Burkitt lymphoma. The air-dried and stained archival FNA smears were destained and analyzed by FISH using a *C-MYC* breakapart probe. In all cases, the diagnosis of Burkitt lymphoma was made on surgical biopsy material obtained subsequent to the FNA.

Results: The FNA biopsies were performed between 1996 and 2004. FNA from all 9 cases showed typical cytologic features of Burkitt lymphoma. Flow cytometry was performed on all 9 FNA cases and demonstrated monoclonal kappa or lambda, CD10 expression in the analyzed cases. FISH was successful in 4 of the 9 cases, all of which showed *C-MYC* rearrangement (44%). Up to100 cells were scored if sufficient numbers of cells were available on the smears. The average positive cells showing *C-MYC* translocation were 82% (71 to 86%). In 5 of the 9 cases (56%), FISH was unsuccessful with no signals detected. Concomitant control smears of a reactive lymph node or Burkitt lymphoma showed appropriately strong FISH signals. The ages of the archival specimens in the FISH successful and failed cases were 3 years (2 to 4 years) and 6 years (3 to 10 years) respectively.

Conclusions: 1) This study shows that detection of *C-MYC* rearrangement by FISH in previously stained cytologic smears is feasible. *C-MYC* rearrangement can be successfully detected in a portion of Burkitt lymphoma cases. 2) FISH is more likely to be successful in relatively recent specimens (within 3 years). Older destained slides are less suitable for FISH analysis possibly due to DNA degradation.

356 Predictive Value Provided by Immunostaining SurePath™ Cervical Specimens with ASC-H Diagnosis for p16^{INK4A} or ProEx™ C

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Background: Approximately 50% of pap smears with the ambiguous diagnosis of atypical squamous cells, cannot high grade dysplasia (ASC-H), are negative for dysplasia (NIL) in follow up colposcopic examination and biopsy. In contrast, high grade squamous intraepithelial lesion (HSIL) pap diagnoses have very high positive predictive value with greater than 99% positive biopsies. Recent studies have shown that strong positive immunostaining for p16^{INK4A}, or ProEx™C supports a diagnosis of dysplasia in surgical biopsies and HSIL pap smears. The objective of our study was to determine whether immunostaining SurePath™cervical specimens for p16 or ProExC provides predictive value that will distinguish high grade dysplasia from atypical squamous metaplasia in ASC-H pap smears.

Design: All ASC-H diagnoses from September 2005 to September 2006 diagnosed at OHSU were identified for analysis (48/9691=0.5%). Residual material from 68 SurePath cytology samples (10 negative (NIL), 48 ASC-H, and 10 HSIL) was processed to produce 3 slides per sample for immunohistochemistry. Cases lacking similar cellularity between slides were excluded from the study (2 NIL and 12 ASC-H). One slide of each sample was stained for either p16, ProExC, and negative control isotype. Slides were scored as positive or negative by two independent cytopathologists (AS and TM) while blinded to pap diagnosis and follow up biopsy data. Immunophenotype was then compared to the pap smear diagnosis and subsequent follow up colposcopic biopsy performed for ASC-H diagnosis (n=17) when available.

Results: We observed excellent agreement in scoring immunostains between investigators (p16 kappa statistic was 0.80; ProExC 0.85). Correlation between p16 and ProExC scores was moderate (kappa 0.50). Chi-square analysis comparing immunophenotype to pap smear outcome showed a strong correlation between positive staining for either p16 or ProExC and HSIL in controls (Fisher's exact P-value <0.001). There was also a significant correlation between positive staining for ProExC and biopsy outcome in ASC-H cases (P<0.05), and a trend towards significance with p16 (p=0.12). Sensitivity and predictive value of ProExC exceeded p16 in these ASC-H cases (*p16*: SN 83, PPV 83, NPV 60; *ProExC*: SN 100, PPV 92, NPV 100).

Conclusions: Immunostaining SurePath pap smears diagnosed with ASC-H for ProExC appears to provide improved predictive value compared to p16 or Bethesda classification alone. Additional biopsy outcome data is required to support this impression.

357 Low Volume Body Cavity Fluids Are Not Truly Low Volume Samples

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Background: Diagnostic challenges arise when evaluating low volume body cavity fluids by limiting sample cellularity, the number of cytopreparations and ability to perform ancillary studies. But are low volume samples received for cytologic examination truly low volume clinical samples? This study was initiated to compare the volume of fluid received in cytology to the volume collected clinically.

Design: A retrospective review was performed on consecutive body cavity fluids received over a 6 month period consisting of 20 mL or less of fluid. The clinical records were reviewed to determine the actual volume collected, gross appearance, presence of simultaneous studies and originating ward of the fluid. The samples were categorized into 3 groups; total volume collected equals that received in cytology, clinical samples < 50 mL (low volume) and clinical samples > 50 mL (high volume).

Results: Over the 6 month interval, 195 body cavity fluids (7 pericardial, 89 pleural, 99 peritoneal) were received with a mean volume: 9.7 mL, range: 0.3 to 20 mL, collected from 184 patients. The volume collected clinically was documented in 74 (37.9%) of the samples (4 pericardial, 36 pleural, 34 peritoneal) from 72 patients. In 2 cases the clinically documented volume of fluid equal that received in cytology. In 14 samples (18.9%), < 50 mL of fluid was collected (low volume) and in 58 samples (78.4%), 50 mL or more was collected (high volume) with 56 cases (75.7%) actually having more than 100 mL. The mean volume of fluid recorded clinically was 1,470 mL (range 1.3 to 9,750 mL). In the low volume category, a mean of 10.8 mL (range 5 to 20 mL) of fluid was received for cytologic examination with a mean of 14.5 mL (range 5 to 32 mL) not received in cytology. In this category, 12 of the 14 samples had additional studies performed on the fluid. In the high volume category a mean of 9.2 mL (range 2 to 20 mL) was received for cytologic examination with a mean of 1,861 mL (range 30 to 9,745 mL) not received in cytology. Only 24 of the 59 high volume samples had additional studies performed. The two source locations of samples in which a large volume of fluid was collected but not provided for cytologic evaluation were diagnostic imaging and the operating rooms.

Conclusions: Approximately 20% of low volume samples received for cytologic examination appear to be the result of recovery of small quantity of fluid clinically and rationing of the sample among various studies. However, in 80% of cases large volumes of fluid are collected, with only a small volume provided for cytologic evaluation.

358 Ultrasound-Guided Fine Needle Aspiration Biopsy of Thyroid Nodules ≤ 1.5 cm, Is It Useful?

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Background: The detection rate of thyroid nodules has increased dramatically due to frequent incidental discovery during ultrasonography (US) as well as MR and CT imaging. The clinical management of patients with palpable solid thyroid nodules > 1.5 cm is well established, however the evaluation of patients with non-palpable small thyroid nodules is controversial. The necessity to perform a fine needle aspiration (FNA) in these cases is a frequent matter of discussion. The aim of this study was to assess the usefulness of ultrasound- guided fine needle aspiration (US-FNA) in the diagnosis of thyroid nodules measuring ≤ 1.5 cm.

Design: A retrospective review of computerized data and charts of 54 patients (46 females and 8 males) with a mean age of 47 years (range 21-73 years) who underwent US-FNA for nodular thyroid disease between June 2001 and August 2006 was conducted. Only patients with available FNA and histologic follow-up were considered. We divided patients into two groups according to the size of thyroid nodules detected by US. Group 1 included 14 patients (11 females and 3 males) with thyroid nodules ≤ 1.5 cm (range 0.8-1.5 cm, mean 1.2 cm). Group 2 included 40 patients (35 females and 5 males) with thyroid nodules >1.5 cm (range 1.7- 8.0 cm, mean 3.6 cm).

Results: The FNA specimens from group 1 were diagnosed as benign (4/14, 28.5%), neoplasm (5/14, 36%), suspicious for carcinoma (1/14, 7%), malignant (0/14, 0%), and non-diagnostic (4/14, 28.5%). The permanent histology specimens from group 1 were diagnosed as benign in 9 nodules (64%) and malignant in 5 nodules (36%). Of the 5 malignant nodules, 3 nodules (60%) were papillary microcarcinoma. Of the 4 cases in the non-diagnostic category from group 1, the histologic follow-up showed papillary carcinoma in two specimens (50%). The FNA specimens from group 2 were diagnosed as benign (14/40, 35%), neoplasm (17/40, 42.5%), suspicious for carcinoma (1/40, 2.5%), malignant (4/40, 10%), and non-diagnostic (4/40, 10%). The permanent histology specimens from group 2 were diagnosed as benign in 25 nodules (62.5%) and malignant in 15 (37.5%). The follow-up histology of the 4 cases in the non-diagnostic category in group 2 was benign.

Conclusions: The results confirm that (US-FNA) is a useful tool in the initial evaluation of thyroid nodules ≤ 1.5 cm, however there is an increased risk of initial non-diagnostic cytology. Non-palpable small thyroid nodules can be clinically significant.

359 FNA of Misclassified Primary Malignant Neoplasms of Thyroid: Impact on Patient Management

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Background: Fine needle aspiration (FNA) is a popular, reliable and cost effective technique for the diagnosis of thyroid lesions. The aim of our study was to review FNA cases of misclassified primary malignant neoplasms of the thyroid and assess its impact on patient management.

Design: We conducted a retrospective review of malignant neoplasms of thyroid diagnosed by FNA. Clinical findings, aspirate smears, histologic slides and management of cases diagnosed with different types of primary thyroid malignancy on histologic follow up were evaluated.

Results: Of the 365 cases with a malignant diagnosis on FNA, 16 (4.4%; Mean-65.8 years; Range 44-84 years; M: F=0.45) were identified with discrepant histologic diagnosis with regard to type of primary thyroid malignancy. Final histologic and initial FNA diagnosis are shown in the table below. Areas of difficulty contributing to misclassification included overlapping cytologic features (n=9), rarity of tumors (n=4), and sampling limitations (n=3). Of the 16 cases, 15 underwent total thyroidectomy and 1 patient had concurrent surgical biopsy. Intraoperative findings resulted in lymph node excision in 2 cases. Initial cytologic diagnosis of medullary carcinoma (with follow up of hurthle cell and papillary carcinoma) resulted in unnecessary lymph node dissection in two cases. Misclassification of medullary as papillary carcinoma precluded lymph node dissection in 1 case. Further management decisions were based on the final histologic diagnosis and did not require additional surgery.

Histologic diagnosis (No. of cases)	Cytologic diagnosis (No. of cases)
Papillary carcinoma (4)	Adenocarcinoma (2), Medullary carcinoma (1), Myxoid liposarcoma (1)
Insular carcinoma (3)	Papillary carcinoma (2), Medullary carcinoma (1)
Hurthle cell carcinoma (3)	Papillary carcinoma (1), Medullary carcinoma (1), Follicular carcinoma (1)
Follicular carcinoma (2)	Papillary carcinoma (1), Poorly differentiated carcinoma (1)
Anaplastic carcinoma (2)	Papillary carcinoma (2)
Medullary carcinoma (1)	Papillary carcinoma (1)
Anaplastic plasmacytoma (1)	Large cell lymphoma (1)

Conclusions: A small fraction of primary malignant neoplasms of the thyroid may be misclassified with regard to the type of malignancy on FNA. The majority of primary malignant neoplasms diagnosed on FNA require thyroidectomy. However, initial cytologic misclassification of medullary carcinoma as other malignant neoplasms or vice versa may have an impact on the decision to perform a lymph node dissection.

360 Impact of Polyoma Virus on UroVysion™ FISH Results in Atypical Urine Cytologies

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Background: Cytologic features of polyoma (BK) virus in urothelial cells are well recognized, but associated nuclear changes could be regarded as atypical and recent data suggest association of BK and development of urothelial carcinoma (UC). UroVysion FISH has higher sensitivity and equally high specificity vs cytology for diagnosis of UC and is useful in resolving atypical cytologies. The impact of BK virus on FISH in screening or monitoring for recurrence of UC is not known.

Design: Urine cytology cases with BK on which FISH was performed were reviewed. Specimens were submitted for Cyto plus FISH, Cyto and FISH if cyto non-neg, or Cyto only, for which FISH was recommended for non-neg cyto. Cytology and UroVysion (Vysis) were performed on equal volumes of fixed urine, predominantly on ThinPrep (Cytyc) slides. Cytology for 40/62 BK cases that had FISH were blindly reviewed for number, density, and % BK cells and % non-BK atypical cells.

Results: From Jan 04-Aug 06, 56,797 urine cytology and 9036 UroVysion tests were performed. 353 cytologies (0.6%) had features of BK noted prospectively, 62 of which had FISH (20 hx UC, 42 hematuria;1 known hx renal transplant). 0% and 1.1% BK cytos were diagnosed positive and suspicious for UC, respectively, vs 1.2 % and 3.4 % total cases. 56.4% BK cases were diagnosed atypical vs 14.9% total cases. FISH was positive in 0/3 BK cases with negative cyto and 7/58 (12%) BK cases with atypical cyto, all by polysomy (vs ~25% in total cases with atypical cyto). Mean/median BK cells/cyto slide were 24.6/11 (range, 2-309). 19/39 (49%) atypical BK cases with FISH did not have any non-BK atypical cells on cyto, and positive FISH cases with atypical cyto included some without non-BK atypical cells on cyto. There was no difference between FISH neg and FISH pos atypical BK cases in number or % of BK cells or presence of non-BK atypical cells.

Conclusions: Urine cytologies with BK were more likely to be diagnosed atypical, but 12% positive UroVysion was lower than non-BK atypical cytologies. No FISH negative BK cases with follow-up had UC on cystoscopy. For positive BK FISH cases, whether BK cells are the population with FISH genetic changes remains to be studied. Higher numbers will be required to address possible false or anticipatory positive rates in BK vs usual cases. Given high positive predictive value of UroVysion for recurrent and new UC, cytology cases with atypical BK cells should likely undergo further evaluation, including FISH.

361 The Immunohistochemical Expression Pattern of SMAD4, p53, and CDX2 Is Helpful in Diagnosing Pancreatic Ductal Adenocarcinoma in Endoscopic Ultrasound-Guided Fine Needle Aspirations (EUS-FNA)

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Background: Cytological distinction between ductal adenocarcinoma of the pancreas and chronic pancreatitis in EUS-FNA specimens can be difficult due to limited cellularity, subtle morphological features in well-differentiated adenocarcinoma, and frequent gastrointestinal (GI) epithelial contamination. SMAD4/DPC4 and p53 are tumor suppressor genes shown to be genetically inactivated in over 50% of ductal adenocarcinomas of the pancreas. Therefore, we analyzed the diagnostic utility of these two markers, in combination with CDX2, a marker for GI epithelial differentiation, in pancreatic EUS-FNA samples.

Design: 33 pancreatic EUS-FNAs with a positive diagnosis of ductal adenocarcinoma with surgical and/or clinical follow-up and 7 histologically confirmed cases of chronic pancreatitis were identified. Immunostians for SMAD4, p53, and CDX2 were performed in 22 adenocarcinoma and 2 chronic pancreatitis cases with cell block materials. The immunostaining pattern was scored as negative (0%), partial staining/loss (0-50%), and diffuse staining/loss (over 50%) based on the percentage of positive cells.

Results: 17 of 22 adenocarcinoma cases showed interpretable results, and tumor cells demonstrated a loss of SAMD4 expression in 76% with complete loss in 47% (8/17) and partial loss in 29% (5/17). Positive p53 was detected in 65% with diffuse staining in 47% (8 /17) and partial staining in 18% (3/17). No staining for CDX2 was found in tumor cells (0/17). By contrast, the GI epithelial cells found in 4 of 17 cases showed expression of CDX2, intact expression of SMAD4 and negative p53. In addition, the ductal and acinar cells in 2 chronic pancreatitis cases showed intact expression of SMAD4, negative p53 and variable staining for CDX2.The combined expression patterns of SMAD-4 and p53 in tumor cells can be categorized into four groups: loss of SMAD-4 with positive (53%) or negative (23%) p53 and intact SMAD4 expression with positive (12%) or negative (12%) p53, while the benign epithelial cells showed uniformly intact SMAD4 expression with negative p53 (100%).

Conclusions: The loss of SMAD4 expression and accumulation of p53 are frequent events in pancreatic ductal adenocarcinomas and can be detected in cytological specimens by immunohistochemistry. The combined expression pattern of loss SMAD4 with positive p53 is diagnostically useful in differentiating ductal adenocarcinoma from a reactive process, like chronic pancreatitis.

362 Fine Needle Aspiration of Abdominal Fat Pad for Diagnosis of Early Amyloidosis: How Can the Clinical Role of the Test Be Improved?

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Background: Many studies have reported fine-needle aspiration of abdominal fat pad as a simple tool for the diagnosis of amyloidosis. However, depending on indication, the methods for detecting amyloid may vary. In this study, we evaluated the practical and optimum approach of ruling out disease in early amyloidosis.

Design: We retrospectively analyzed 36 abdominal fat pad aspirates (2 insufficient for evaluation) from 34 cases performed and interpreted by more than 9 cytopathologists over a period of 5 years. Majority of aspirates were performed to rule out early disease secondary to monoclonal gammopathy. Based on retrospective clinical details, 9 cases had amyloidosis. 34 adequate specimens were evaluated by studying 10 µm thick Congo red stained cell block sections under polarized microscopy. From 24 aspirates, representative specimen was also submitted simultaneously for ultrastructual studies.

Results: 44% cases (4 of 9) with amyloidosis could be diagnosed by ultrastructural study of fat pad aspirate. These 4 aspirates were performed and interpreted by 3 cytopathologists. All 34 cases, except 1 with advanced amyloidosis, evaluated with Congo red stained cell block sections alone were interpreted as negative. To evaluate interobserver reproducibility, Congo red stained cell block sections from 4 positive cases were mixed with 8 negative cases. One of the cases with autopsy confirmed advanced amylodosis with cardiomyopathy, and positive with ultrastructural study, was interpreted positive with Congo red by 3 of 4 pathologists (1 equivocal). However, these pathologists also interpreted a few negative cases as positive. The results among four pathologists showed variable sensitivity (25-75%) and specificity (50-100%). Kappa index 0.1273 (95% CI -0.1037 to 0.3583) for multiple observers was consistent with lack of agreement by Congo red alone.

Conclusions: 1. Evaluation of Congo red stained sections under polarizing microscope alone had poor interobserver reproducibility and it underdiagnosed early amyloidosis in fat pad aspirates. 2. Routine ultrastructural evaluation of fad pad aspirates is recommended for diagnosis of early amyloidosis.

363 Stromal Foam Cells in Endometrial Smears: A Diagnostic Clue to Endometrial Adenocarcinoma

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Background: It has been reported that stromal foam cells (SFCs) are sometimes observed in cases with endometrial atypical hyperplasia and endometrial adenocarcinoma in endometrial smears as well as histological specimens. Although endometrial smears are not popular in the U.S.A., in some countries it is commonly used to detect endometrial adenocarcinoma, and its diagnostic level has recently been improved. To our knowledge, however, the differences between SFCs and foam cells in glandular lumina (luminal foam cells, LFCs) as well as the significances of these cells have not yet been fully evaluated.

Design: Histologically diagnosed 16 cases of endometrioid adenocarcinoma (EA)(FIGO, grade 1), 12 cases of complex hyperplasia (CH), 23 cases of complex atypical hyperplasia (CAH), and 15 cases of normal endometrium were randomly archived (total; n=66) from our hospital file. To evaluate the correlation between foam cells and the conditions mentioned above, both the histological sections and cytological smears of the endometrium were investigated.

Results: In histology, SFCs were identified in 8 of 66 cases, and all of them had EA. On the other hand, LFCs were observed in 13 cases in total, and with EA (n=4), CH (n=3), and CAH (n=6). On cytological smears of the endometrium, while the cytoplasm of SFCs was uniform and finely vacuolated, LFCs contained distinct vacuoles in various sizes. SFCs were found mainly among loose aggregates of epithelial cells or the edge of cohesive epithelial clusters. On the other hand, LFCs were not particularly related to epithelial cells, and occurred as isolated cells. Four out of 8 cases with SFCs in histology showed these cells on cytological smears. Among 13 cases with LFCs in histology, 11 cases had LFCs, and none of them revealed SFCs on cytological smears of the endometrium.

Conclusions: Our study indicates that the presence of SFCs, which is found among loose agregates of epithelial cells or the edge of cohesive epithelial clusters, on endometrial smears can alert cytopathologists as to the possibility of EA. SFCs may be a diagnostic clue to EA. Once the pathologist becomes accustomed to the cytological findings of SFCs, it is easy to differentiate between SFCs and LFCs. Since the diagnosis of endometrial adenocarcinoma in endometrial smears may be underdiagnosed, the possibility of SFCs in addition to ordinary cytological findings of adenocarcinoma in endometrial smears should be kept in mind.

364 Clinico-Pathologic Characterization of "Thyroid Bed" Fine Needle Aspiration

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Background: Fine needle aspiration (FNA) of thyroid bed (TB) lesions is an important diagnostic modality in the management and monitoring of patients for recurrent/residual cancer who are status-post thyroidectomy.

Design: We reviewed all FNAs of TB from our cytopathology archives for a 17-year period (1989-2006). Search criteria included only cases that were specifically in the TB; cases in the surrounding neck and nearby lymph nodes were excluded.

Results: Thirty-four FNAs were identified with a mean age of 52.9 years (range 25-81) and a M:F ratio of 1:1.78. The average size of the TB nodule was 1.7 cm (range 0.4-5). TB nodule locations were left side (15, 44%), right side (17, 50%), midline (1) and bilateral (1). Diagnoses at thyroidectomies were papillary carcinoma [PC] (21), medullary carcinoma [MC] (8), papillary microcarcinoma in multinodular hyperplasia (1), Hurthle cell carcinoma [HCC] (1), follicular carcinoma [FC] (1), poorly differentiated neuroendocrine neoplasm [PDNE] (1), and multinodular hyperplasia (1). Of the 25 cases where the original side of the neoplasm was known, 12 (48%) cases had TB nodules appearing on the ipsilateral side, 10 (40%) cases had nodules lateralizing to one side following a primary bilateral or midline lesion, 1 (4%) case showed bilateral nodules from a unilateral primary, and 2 (8%) cases had TB nodules on the contralateral side. Recurrent disease demonstrated the expected cytomorphologic features. The average interval between initial thyroidectomy and FNA of the TB nodule was 99 months (range 2.6 to 341). In 19 (56%) of 34 FNAs, the final diagnosis was PC, while MC was present in 6 cases (18%), and PDNE was seen in 1 case (3%). The remaining 8 cases included benign and unsatisfactory aspirates: three of benign residual thyroid tissue (9%), three of benign lymphoid tissue (9%), and two of predominantly blood (6%).

Conclusions: TB FNA found recurrent disease in 17 of 19 (89%) where PC was the initial diagnosis. A limited sample demonstrates that TB nodules are less likely to show recurrence in MC (4 of 6), papillary microcarcinoma (0 of 1), HCC (0 of 1), and FC (0 of 1). As in the primary diagnosis of thyroid nodules, FNA of TB masses remains an important tool in the follow-up care of patients with thyroid neoplasia. The threshold for diagnosis of malignancy in TB lesions is lower than for primary diagnosis and is often made with scant or minimal material.

365 Use of Hyperspectral Imaging in Detection of Precancerous Cells

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Background: Traditional pathologic evaluation of cells and tissues is via light microscopy utilizing only visual range (400-700 nm) of the light spectrum. Using Hyperspectral Imaging (HI) (400-1000 nm) we successfully distinguished normal, precancerous low grade and high grade cervical cells in liquid Papanicolaou test slides, through newly developed algorithms utilizing spectral ratios, spectral and spatial differences among the cell nuclei. Within the next few years, this approach in conjunction with slide scanners may assist us in automated detection of precancerous and cancerous cells.

Design: A Nikon Eclipse 800 (Nikon Corporation, Tokyo, Japan) upright microscope equipped with a V100-E hyperspectral camera (ProVision Technologies, Stennis, Mississippi) was used to acquire HI scans of normal and precancerous cervical cells on Tripath® (Burlington, NC) liquid Papanicolaou test slides. The algorithms developed in our previous study for differentiating normal fibroblasts, telomerase transformed and SV 40 transformed fibroblasts, which were selected as an analogy to normal, low grade and high grade precancerous cervical cells were then used with slight modifications to differentiate the above.

Results: The system identified normal cells with 95.83% sensitivity and 94.46% specificity, low grades cells with 66.67% sensitivity and 99.21% specificity and high grade cells with 93.48% sensitivity and 88.37% specificity.

Conclusions: The above results show the potential of HI use in pathology. HI can be utilized in prescreening liquid Papanicolaou slides to improve accuracy in Papanicolaou test diagnosis. We are currently working to further refine this methodology. The ultimate use of HI as an ancillary study like immunostains and gene rearrangement in tumor diagnosis is also under consideration.

366 The Utility of SMAD4 as a Diagnostic Immunohistochemical Marker for Pancreatic Adenocarcinoma, and Its Expression in Other Solid Tumors

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Background: Pancreatic adenocarcinoma is a genetic disease showing somatic mutations of multiple genes, including SMAD4. It is a tumor suppressor gene that is inactivated in a sub-set of pancreatic adenocarcinomas, either by the intragenic mutation of one allele in combination with the loss of the other allele or by homozygous deletion of both alleles. In the cytoplasm, SMAD4 protein mediates signals from a family of TGF-ß ligands and their transmembrane receptors through phosphorylation of SMAD4 proteins, which heterodimerizes with SMAD4. This SMAD4/SMAD complex transmits upstream signals by translocating to the nucleus, binding to specific DNA sequences, and activating gene transcription. Many of the functions of TGF-ß and its ligands, such as growth suppression and apoptosis are abrogated by inactivation of SMAD4. This study examines SMAD4 expression in fine needle aspiration cell blocks from patients with pancreatic adenocarcinoma, as well as a variety of human cancers, in order to assess its viability as a tumor marker.

Design: A total of 100 patients with pancreatic adenocarinoma, with diagnostic material from fine needle aspiration cell blocks were selected for this study. In addition, cancers from different sites were examined in multitumor tissue microarrays, which included two tissue cores from neoplastic specimens. Cancers studied included endometrium (n=100), lung (n=100), colon (n=100), ovary (n=100), and melanoma (n=100). Sections, were immunostained with SMAD4 using pressure cooker antigen retrieval labeled polymer HRP (DAKO), and the DAKO autostainer. Immunohistochemical expression was scored as negative, 1+, 2+, and 3+. Only 2+ and 3+ staining was considered as positive staining.

Results: SMAD4 staining was nuclear and the results for tumor cell positivity for primary sites studied, are as follows: Pancreas (80/100; 80%), endometrium (0/100; 0%), breast (2/100; 2%), lung (0/100; 0%), colon (0/100; 0%), ovary (3/100; 3%), melanoma (4/100; 4%).

Conclusions: This study suggests that SMAD4 is an important marker for confirming a diagnosis of pancreatic adenocarcinoma. SMAD4 expression is absent in endometrial, lung and colon carcinomas. A very small sub-set of breast and ovarian carcinomas, and melanomas may show SMAD4 expression. In summary, this study indicates that SMAD4 can be helpful in confirming the diagnosis of pancreatic adenocarcinoma, as a primary tumor, as well as when it presents as a metastatic tumor on small fine needle aspirate samples.

367 The Cytopathologist's Role in Chemo-Response Testing

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Background: Chemo-response testing is now being performed at commercial laboratories to better assist oncologists in selecting the appropriate chemotherapeutic regimen for treating cancer patients. Obtaining an enriched malignant epithelial cell population is required for *ex vivo* chemo-response prediction. Determining the integrity of the malignant cells in primary culture is essential for this testing. Currently, immunofluorescent (IF) studies are done to determine if a malignant epithelial cell population rather than fibroblastic overgrowth is present. We evaluated whether the cytopathologist's examination applying conventional cytomorphologic criteria for epithelial differentiation can be useful in determining the presence of malignant cells in the initial culture and cellular enrichment in short-term primary cultures for chemo-response studies.

Design: Fifty human tumor specimens (breast, colon, lung, ovary) were established and maintained in short-term primary culture. Cytospins of the cell suspension were prepared upon the initiation of the culture and at the completion of the culture process. The smears were stained with Diff-Quik and Papanicolaou stain, and evaluated by a cytopathologist using cytomorphologic criteria for malignancy at both time points. The % of malignant cells in the cultured cell population that could potentially also include fibroblasts and/or inflammatory cells was determined.

Results: All of the culture specimens reached at least 60% malignant epithelial cells by the end of the culture period based on cytomorphologic features. The majority of the specimens (86%) showed enrichment for malignant cells, while 8% remained the same and 6% showed decreased numbers of malignant cells. Using the commercial lab IF positivity of equal or greater than 65% of the cells staining positive for one or two epithelial antigens (pancytokeratin AE1/3 and Cam 5.2), cytomorphologic assessment had 45 cultures passing with 1 IF failure and 4 cytomorphologic failures with 0 IF failures.

Conclusions: Pathologists are increasingly involved in providing chemotherapeutic response prediction of malignancies in routine clinical practice. Chemo-response testing is now also being utilized for the selection of the most appropriate chemotherapeutic agents to treat a variety of malignancies. We believe that the cytopathologist has an important role in assessing epithelial malignancy viability for chemo-response testing by using standard cytomorphologic criteria of malignancy of cells cultured for chemo-response testing.

368 Correlation of Fine Needle Aspiration Biopsy Outcomes with CT and PET Scan Findings of Non-Calcified Solitary Pulmonary Nodules Identified in Patients with Extrapulmonary Malignancies

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Background: Solitary pulmonary nodules (SPN) are frequently discovered in patients undergoing CT scan surveillance. PET scanning is currently considered as a modality of choice in investigating such nodules due to its non-invasive nature. Overall aim being to identify malignant lesions early at the same time avoid unnecessary morbidity of invasive procedures in patients with benign lesions. Our aim was to correlate the outcomes of fine needle aspiration biopsy (FNAB) with preoperative CT and PET scans in a cohort of patients with non-calcified SPN identified in patients undergoing surveillance for non-pulmonary malignancies.

Design: Retrospective data from 87 patients who underwent CT scan guided biopsies for incidental SPN (less than 3 cm) are included in this study. In all cases the pulmonary nodules were discovered during surveillance for a non pulmonary malignancy. Preoperative CT scans and PET scans were reviewed. The CT scans classified as suspicious or indeterminate nodules were considered as positive by CT scan for statistical analysis. All lesions with a standard uptake value of 2.5 or more were considered PET positive for malignancy. Sensitivity, specificity, negative predictive value and accuracy was calculated for each procedure (FNAB, CT and PET scan). The gold standard for determining sensitivity was a diagnosis based on histopathologic analysis.

Results: Overall, by histopathology there were 75 cases with malignancy (60 primary, 15 metastatic tumors); the remaining 12 cases were benign. The FNAB diagnosis were benign in 17 and malignant in 70 cases (false negative in 5 cases) [sensitivity 93%, specificity 100% and negative predictive value 70%]. CT scan results were positive in 58 cases [sensitivity 69%, specificity 54% and negative predictive value 20%]. PET scan was positive in 40 cases (34/48 primary tumors and 4/11 metastatic tumors) [sensitivity 64%, specificity 75% and negative predictive value 22%].

Conclusions: PET scan was only moderate sensitive with a low negative predictive value in this study. Specifically PET scan was positive in only 36% metastatic tumors. For such patients FNAB was the most accurate and sensitive procedure. Our results suggest that a multidisciplinary approach should be used in evaluations of solitary pulmonary nodules in this high risk population with high pretest probability of malignancy.

369 Cytology Specimens Compare Favorably with Surgical Specimens for *EGFR* Mutation Detection in Patients with NSCLC

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Background: Somatic mutations in the epidermal growth factor receptor (*EGFR*) are present in 10-15% of non-small-cell lung cancers (NSCLC). NSCLC with these oncogenic mutations respond clinically to treatment with tyrosine kinase inhibitors (TKIs, gefitinib, erlotinib). Two mutations are common- a small in-frame deletion in exon 19 (45%) and an L858R point mutation in exon 21 (35%) - but other significant mutations have been reported. In order to detect all possible significant *EGFR* mutations, direct sequencing of tumor DNA is used, which is limited by interference from nonmalignant cells in the specimens, requiring manual microdissection to enrich the sample for cancer DNA before analysis. Concern over this interference has discouraged the testing of cytology samples, such that they have been used mostly when no adequate surgical material was available. This study aimed to determine whether or not cytology samples are suitable for *EGFR* sequencing.

Design: *EGFR* sequencing of surgical and cytology specimens at Brigham and Women's Hospital over the past two years was reviewed. 239 cases were analyzed, of which 227 were surgical specimens and 12 (5%) were cytology specimens, including FNAs, pleural fluids, bronchial washings, and BALs.

Results: Of the surgical specimens, 63 (27.8%) were positive for *EGFR* mutations, 143 (62.6%) were negative, 8 (3.5%) failed to amplify, and 14 (6.2%) were inconclusive (a negative result in a highly heterogeneous sample). Of the cytology specimens, 7 (58.5%) were positive for *EGFR* mutations, 4 (33%) were negative, and 1 (8.3%) was inconclusive. Cytology specimens showed significantly higher sensitivity for detecting mutations compared to surgical specimens (p=0.02). There was no significant difference in the frequency of inconclusive results. Mutations were detectable in cytology samples with as little as 25% tumor cellularity (of total nucleated cells).

Conclusions: Cytology specimens are suitable for *EGFR* sequencing and show higher sensitivity for mutation detection than do surgical specimens. Heterogeneous cytology specimens with modest tumor cellularity can be used for mutation detection (as low as 25% tumor cellularity in our study). The suitability of a sample should be determined on a case-by-case basis, and cytology samples should not be dismissed as inadequate without a thorough review.

370 Sensitivity and Specificity of Polyoma Viral Cytopathic Effect in Urine Cytology Samples as a Function of Viral Load as Determined by Quantitative PCR for BK Virus

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Background: Since BK viruria in stem cell allograft patients appears to be linked to hemorrhagic cystitis, quantitative PCR has become a popular send out test at our institution. Many of these patients also have a concurrent urine sample sent to Cytology for morphologic detection of polyoma virus cytopathic effect. Reviewing these cases led us to ask: a) What is the viral load below which viral cytopathic effect cannot be observed? b) How good of a screening test is urine cytology above that threshold? c) What is the clinical significance?

Design: The results of 122 quantitative PCR assays for urine BK virus having a concurrent urine sample submitted to Cytology for detection of polyoma viral cytopathic effect were retrospectively studied. Only the cytology results were reviewed; the slides were not re-examined. The BKV load and corresponding urine cytology results were tabulated. Urine cytologies reported as "consistent with" or "suspicious for" were considered positive. The sensitivity, specificity, positive predictive value and negative predicitive value for urine cytology were calculated as a function of viral load.

Results: No cytopathic effect was identified by Cytology in any case having a viral load below 2 x 10^6 virons/ml.

Table 2. Detection of Polyoma Virus Cytopathic Effect in Urine

	BK-Viral Load by Quantitative PCR (in 10^6 virons/ml. urine)						
	≥200	≥20	≥2	≥0.2	≥0.02	≥0.002	≥0.0002
TP	24	28	29	29	29	29	29
FN	9	8	10	16	23	32	44
FP	3	3	2	2	2	2	2
TN	86	83	81	75	68	59	47
P_{PCR}	33	36	39	45	52	61	73
N_{PCR}	89	86	83	77	70	61	49
P_{Cyto}	27	31	31	31	31	31	31
N_{Cyto}	95	91	91	91	91	91	91
Sens%	73	78	74	64	56	48	40
Spec%	97	96	98	97	97	97	96
(+)PV%	89	90	94	94	94	94	94
(-)PV%	90	91	89	82	75	65	52

Conclusions: The threshold for detecting polyoma virus in urine was approximately 2 x 10^6 virons/ml. Above this level, urine cytology had a sensitivity and specificity of 74% and 98%, respectively with Positive and Negative Predictive Values of 94% and 89%, respectively. Since most studies identify a viral load above 10^7 virons/ml. to be evidence of viral reactivation, the results of this study indicate that urine cytology should be considered a useful screening test for viral reactivation in known BK virus carriers.

371 An Immunocytochemical Battery for the Distinction of Submucosal Gastrointestinal Mesenchymal Neoplasms Sampled by EUS-Guided FNA

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Background: Endoscopic ultrasound-guided fine needle aspiration (EUS-guided FNA) has been shown to be a safe and effective method for the diagnosis of gastrointestinal (GI) and adjacent lesions, including submucosal GI mesenchymal neoplasms (SGIMNs). The distinction of the various types of mesenchymal neoplasia can be important as these lesions may carry different prognoses and may be amenable to different treatments (e.g., gastrointestinal stromal tumors (GISTs) can be treated with tyrosine kinase inhibition). Although retrospective studies have described the cytologic features and immunocytochemical (ICC) profiles of the various lesions, we are not aware of a large study that investigated the positive predictive value of a limited ICC battery. This study reviews our experience with the use of ICC applied to samples of SGIMNs sampled by EUS-guided FNA.

Design: Our cytology database was searched for all SGIMNs sampled by EUS-guided FNA with ancillary ICC. All samples were collected by gastroenterologists with on-site interpretation by a pathologist who triaged tissue toward cell block preparation. All ICC results were recorded and compared to final histopathologic diagnoses when available.

Results: Eighty-nine SGIMNs that were sampled by EUS-guided FNA had ICC results. The majority of cases were GISTs (44), smooth muscle tumors (SMTs) (35) and peripheral nerve sheath tumors (PNSTs) (6). ICC results are summarized in table 1.

Table 1

Diagnosis	CD117	CD34	SMA	Desmin	S100
GIST	34/35	37/39	1/36	0/11	0/35
SMT	0/34	0/29	34/34	12/12	0/26
PNST	0/6	1/6	1/5	0/4	6/6

On follow-up, all CD117 and/or CD34 positive tumors that lacked significant immunoreactivity with antibodies to smooth muscle antigens (SMAs) and S100 were GISTs. Aside from single glomus tumor that showed distinct cytology, all lesions which showed strong immunoreactivity with antibodies to SMAs did not show significant reactivity with antibodies to CD117, CD34 or S100 and, at follow-up were SMTs. Finally, all cases showing strong immunoreactivity with antibodies to S100 were, at follow up, PNSTs.

Conclusions: A small battery of ICC stains including CD117, CD34, SMA, and S100 may be all that is required to correctly prospectively diagnose most SGIMNs sampled by EUS-guided FNA. Occasional cases may require other stains depending upon the differential diagnosis.

372 Cytologic Triage of Oral Lesions Using Liquid Based Methodology

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Background: The routine cytologic evaluation of several mucosal sites including the cervix, urinary bladder and esophagus exploits the exfoliative nature of these mucosae. Cells shed or mechanically removed from these accessible surfaces are efficiently and reproducibly analyzed using monolayer techniques. Carcinomas of the oral cavity are a significant cause of morbidity and mortality in the adult population. Direct visualization has been the customary screening tool, but once a lesion is identified, an invasive biopsy or observation without objective data are the only clinical choices. Cytologic brushings may provide tangible information to support a clinical decision to observe rather than biopsy or excise a lesion.

Design: Given that the liquid based methodology has been shown to be superior to conventional smear, we applied the monolayer technique using the ThinPrep® (Cytyc Corporation Marlborough, Massachusetts) technology to brushings of oral cavity lesions. We independently evaluated brushings of oral lesions followed by review of the concurrent surgical biopsy to determine whether the monolayer technique is useful in the triage of epithelial lesions. Cytologic specimens were viewed primarily, followed by review of the biopsy material.

Results: A total of 18 cases were evaluated. The comparative findings demonstrated excellent correlation for 13 epithelial lesions (7/7 benign, 5/5 squamous cell carcinoma and 1/1 with koilocytes/HPV changes). We were not able to detect 5 benign submucosal lesions, although the overlying epithelium was accurately evaluated as benign or reactive.

Conclusions: The performance of cytologic brushings is a useful, easily accomplished, non-invasive technique. The liquid-based monolayer methodology is superior for pathologic evaluation of cytologic specimens. Used together, they show promise as adjuncts to clinical judgment in the decision to follow an epithelial lesion in the oral cavity or proceed to biopsy.

373 Comparison of Pap Test Diagnoses and Reflex High-Risk HPV Testing Results before and after Implementation of Computerized Imager-Assisted Screening

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Background: Accurate screening of Pap tests to detect squamous intraepithelial lesions of the uterine cervix is essential to the practice of cytopathology. Previously, Pap test diagnoses were rendered after manual screening alone. In March of 2005, the ThinPrep Imaging System (Cytyc Corp., Boxboro, MA) was implemented to aid in the screening process.

Design: The computerized database from Vanderbilt University Medical Center was searched for all liquid based Pap tests from the six month period before and after implementation of the computerized imager. The rates of Bethesda diagnoses of atypical squamous cells of undetermined significance (ASCUS), atypical squamous cells of undetermined significance cannot exclude high grade squamous intraepithelial lesion (ASC-H), low grade squamous intraepithelial lesion (LSIL) and high grade squamous intraepithelial lesion (HSIL) were determined. In addition, the ASC/SIL ratio and rates of high-risk HPV positivity in ASCUS cases were calculated.
Results: A total of 8752 Pap tests was examined before introduction of the imaging system compared to 8605 after. For manually screened Pap tests, the diagnostic rates were: ASCUS 5.4%(n=476), ASC-H 0.59%(n=52), LSIL 6.6%(n=577) and HSIL 2.7%(n=232). For Pap tests screened with the imaging system, the diagnostic rates were: ASCUS 6.8% (n= 584), ASC-H 0.51%(n=44), LSIL 6.6%(n=570) and HSIL 2.5%(n=211). The ASC/SIL ratio for the manually screened Pap tests was 0.65, while that of the imaged Pap tests was 0.80. Reflex high-risk HPV testing was performed on 424 cases screened manually with a diagnosis of ASCUS, and on 428 cases screened with the imager. The rate of high-risk HPV positivity was 37.0% (157/424) for cases screened manually and 31.8% (136/428) for cases screened with the imager.
Conclusions: Introduction of the ThinPrep Imaging System in a university teaching hospital setting did not significantly change the diagnostic rates in screening Pap tests when compared to slides screened manually. The ASC/SIL ratio, however, increased from 0.65 to 0.80 with introduction of the imager. High-risk HPV positivity rates on reflex testing of ASCUS Pap tests decreased from 37.0% in manually screened slides to 31.8% after the introduction of the imager.

374 Fine Needle Aspiration of Sacral and Pre-Sacral Lesions: Cytopathologic Analysis and Clinical Correlates

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Background: Lesions of the sacral and pre-sacral area are uncommon and may arise from soft tissues or bone. Diagnostic issues arise on fine needle aspiration (FNA) due to the rarity and difficult anatomic location of these lesions.
Design: A retrospective study (1989-2006) of 91 consecutive sacral and pre-sacral FNAs from a major tertiary care centre was performed. FNAs were done under computed tomography (CT) or ultrasound guidance. Clinical data, radiologic findings and follow-up histopathologic material was additionally reviewed and correlated.
Results: Of the 91 cases, 62% had a prior history of cancer. The most common clinical presentation was lower back pain (44%) and motor and/or sensory dysfunction of the lower extremities (35%). Radiographic imaging revealed lytic bone lesions and/or soft tissue masses ranging in size from 1 to 12 cm (mean - 5 cm). The most common clinicoradiographic impression was metastatic disease. Twenty of the ninety one (22%) cases were non-diagnostic consisting primarily of blood. Of the 71 diagnostic cases, 19 (27%) represented non-neoplastic lesions, 2 (3%) were reported as suspicious for a neoplasm, while 50 (70%) cases were neoplastic. Of the 19 non-neoplastic cases, the most common diagnosis was non-specific acute/chronic inflammation-10 (53%). Two of the fifty neoplastic lesions (4%) were benign tumors (one each of schwannoma and neurofibroma). The remaining 48 (96%) cases were malignant [11 (23%) – primary, 37 (77%) – metastatic/secondary]. The most common primary malignant tumor was chordoma (4/11, 36%) followed by myxopapillary ependymoma (2/11, 18%) and chondrosarcoma (2/11, 18%). Of the 37 metastatic/secondary tumors, the most common primary sites included colorectum (8, 22%) and breast (3, 8%). The most common tumor types were adenocarcinoma (15/50, 30%) and renal cell carcinoma (3, 6%). Secondary tumors (9/37, 24%) consisted of plasma cell myeloma (5/9, 56%), lymphoma (3/9, 33%) and leukemia (1/9, 11%). The overall accuracy, sensitivity and specificity of FNA were 92%, 95% and 100% respectively.
Conclusions: 1) Sacral and pre-sacral lesions represent rare targets for FNA. 2) Although a neoplastic lesion is most commonly encountered, benign tumors are exceedingly rare (2%) with the majority of the lesions being malignant (53%). 3) Most cancers are metastatic (41%), with colorectum being the most common primary site (9%). 4) Primary cancers are rare (14%), with chordoma being the most common tumor type (4%). 5) The overall diagnostic accuracy is 92% with no procedure-related complications.

375 Comparison of Pap Test Five-Year Retrospective Reviews for HSIL and ASC-H

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Background: Current federal regulations require that cytopathology laboratories perform documented five-year retrospective reviews on all negative Pap tests after an HSIL or a malignant lesion is first detected. Similar retrospective reviews for ASC-H cases are not mandated. The purpose of this study is to compare retrospective review results on Pap tests with ASC-H interpretations to those of HSIL.
Design: The computer database files were searched to identify Pap tests with ASC-H or HSIL intepretations for a one-year time period. All ASC-H cases with prior Negative for Intraepithelial Lesion or Malignancy (NILM) Pap test interpretations were retrieved and reviewed by a cytotechnologist and cytopathologist. Discrepancies were documented as either screening/location or interpretive when Pap test interpretations varied from the initial interpretation. Retrospective reviews for HSIL occur routinely in the laboratory. When a Pap test is interpreted as HSIL, the cytotechnologist retrieves all previous NILM cases, reviews them, submits them to the pathologist who interpreted the HSIL case and the pathologist documents any discrepancies. Follow-up was obtained on all discrepant cases.
Results: Of 62,047 Pap tests performed in 2005, 278 were ASC-H (0.44%) and 226 were HSIL (.36%). Of 278 ASC-H cases, 89 patients had prior NILM Pap tests. A total of 156 NILM cases were reviewed on these 89 patients. Twenty discrepancies were identified of which 13/89 (14.6%) had clinically relevant (CIN 1+) follow-up. Eleven of the discrepancies (55%) were screening/location and nine (45%) were interpretive. In contrast, 64/226 HSIL patients had prior NILM cases available for review. A total of 115 Pap tests were identified from these 64 patients with 19 discrepancies identified (16.5%). Of cases with discrepancies, 9/64 had clinically relevant (CIN1+) follow-up (14.1%). Of the 19 discrepancies on HSIL retrospective reviews, 10 (53%) were screening/location and nine (47%) were interpretive.
Conclusions: When comparing retrospective review results of ASC-H patients to HSIL patients, a similar percentage of discrepancies that resulted in clinically relevant follow-up was seen. Screening/location and interpretive discrepancies contributed almost equally as causes of discrepancies in both cases. Additional study will help to determine if restrospective reviews of ASC-H cases is a helpful quality assurance tool in the cytopathology laboratory setting.

376 Correlation of Upper Urinary Track Routine Cytology (RC) and Fluorescence In Situ Hybridization (FISH) Findings with Biopsy Results Including FISH

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Background: The ability to establish pathology in the upper urinary track (UUT) including ureter and renal pelvis is challenging due to limitations in direct visualization of the region of concern. Compounding the problem is the difficulty in interpreting RC from urine samples collected from this location. The utility of FISH is well-established for determining bladder pathology from cells collected from urine. The purpose of this study was to determine the potential role FISH may play in UUT pathology.
Design: 8 cases from 7 patients were evaluated in which the following information was established: Results from RC, FISH cytology (FC), follow-up biopsy (bx), and FISH on bx (FB). The FISH UroVysion™ probe set was used on both urine and follow-up bx material, and specimens were considered positive by FISH if ≥ 5 cells demonstrated gains of two or more signals (polysomy) or if ≥ 20% of cells demonstrated homozygous 9p21 deletion.
Results: There were 7 males and 1 females. The mean age was 71 years (range 51-87). There were 5 from the left ureter, 1 from the right ureter, 1 from the right renal pelvis, and 1 from the left renal pelvis. RC was negative (4), positive (2), or suspicious (2). FC was negative (1; <5 polysomic cells), equivocal (1; 5 polysomic cells), or positive (6; 10 or more polysomic cells). Follow-up bx was negative (2) and positive (6). Of the positive bxes, all were grade 3 (of 3) urothelial carcinomas (1 Ta, 2 Tis, and 3 T1). Of the 4 negative cytology cases, FC correctly established the follow-up bx diagnosis in all cases (2 negative, 2 positive) with complete correlation with FB result. Of the 2 suspicious cytology cases, FC was positive for both with positive follow-up bx and FB results.
Conclusions: FISH using UUT urine specimens may be useful in correctly identifying patients with significant pathology when RC is either negative or equivocal (suspicious). Cut points used for classifying FC in the UUT samples seem appropriate.

377 Dissemination of Toyota Methods To Improve Quality and Patient Safety in Pap Testing

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Background: We previously showed that Toyota Production System (TPS) methods may be used to redesign a single gynecologist's practice and cytology lab service to reduce Pap test error and improve Pap test quality. Our goal was to determine if TPS process redesign may be implemented widely across a group of clinicians and multiple lab personnel to improve cervical cancer screening patient safety.
Design: We performed a one-year non-concurrent interventional cohort study that included 5384 case and 5442 control women who had a Pap test procured by one of five clinicians. We implemented a continuous flow, one-by-one process in the cervical cancer screening practices of 4 gynecologists and 1 nurse practitioner. For each Pap test, the clinicians used a TPS "checklist" that allowed the clinician to focus on every step of Pap test procurement. We assigned a unique cytotechnologist to screen the Pap tests from every clinician, and the cytotechnologists provided immediate feedback regarding specimen adequacy and abnormal results to each clinician. To evaluate adequacy, we measured pre (control) and post (case) implementation proportion of Pap tests with unsatisfactory and absent endocervical transformation zone component (ETZC) interpretations. We also measured the proportion of women with missed and detected cervical intraepithelial neoplasia (CIN) and with a missed squamous intraepithelial lesion (SIL).
Results: After the intervention, the mean proportion of Pap tests lacking a transformation zone component decreased from 17.3% to 15.1% (P = .001) and the proportion of unsatisfactory Pap tests decreased from 1.3% to 0.9% (P = .008). The case group of women showed a 113% increase in newly detected CIN following a previous benign Pap test procured the previous year (P = .004), indicating a missed SIL in the previous year. The case group of women also showed a 16% increase in detected CIN.
Conclusions: Disseminating TPS methods across a group of clinicians and cytotechnologists resulted in improved sampling and fewer false negative diagnoses. TPS redesign resulted in improved communication between the cytology lab and the clinicians and an increased focus on work. Initiatives that target the entire testing pathway help to improve patient safety.

378 Prospective Immunocytochemical Studies of ProEx C as a Biomarker for Cervical Dysplasia in Liquid Based Cytologic Specimens

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Background: ProEx C is a monoclonal antibody cocktail for detection of minichromosome maintenance 2 (MCM2) and topoisomerase II alpha (TOP2A) proteins, over-expressed in a number of different dysplasias and malignancies, such as cervix, There are few published articles discussing ProEx C utility in SurePath liquid based specimens, especially in a prospective methodology. The purpose of this study is to evaluate the accuracy of ProEx C immunocytochemical stain for dysplastic cells in SurePath liquid based Pap test with the correlation of the diagnoses of Pap tests, and surgical biopsies.

Design: 50 consecutive SurePath Pap test specimens were collected and two slides were prepared from each sample; one slide was stained with SurePath Pap stain and the other slide was stained with ProEx C biomarker cocktail. ProEx C biomarkers with SiHa cells as control cells were optimized with SureDetect reagents. Immunocytochemistry study was performed with Dako automated stainer. The SurePath stained slides were routinely diagnosed according to the criteria of the Bethesda System. The ProEx C stained slides were evaluated in a blinded fashion at ten 400 X fields for the number of positive cells, staining intensity, and background staining. The results were correlated and compared with the cytologic diagnoses, any follow-up surgical biopsies and HPV tests.

Results: ProEx C tests showed 30 positive samples out of total 50 SurePath specimens in our study. Compared with the available diagnoses of the paired SurePath Pap tests, high risk HPV results, and surgical biopsies, ProEx C demonstrated 9 positive cases out of 11 HSIL cases (CIN-II and above) with a sensitivity of 82%. The positives generally exhibited intense nuclear staining of positively stained dysplastic cells, but there were a certain number of dysplastic cells unstained. It was of note that there was 54.5% positively stained dysplastic cells of HSIL cell category and only 23.5% positively stained dysplastic cells of LSIL cell category. Endocervical glandular cells, parabasal cells, and occasional histiocytes were frequently positive for ProEx C, which might be a diagnostic pitfall of false positivity.

Conclusions: ProEx C appears to be an excellent potential biomarker for cervical high grade dysplasia. It can be used with combination of routine Pap tests and HPV tests to avoid the false positivity, mainly from parabasal cells and endocervical glandular cells. Further improvement of ProEx C tests and extensive prospective clinical trials may be needed before widespread adoption.

379 ASCUS and Reflex HPV Testing: Correlation with Age and Biopsy Outcome

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Background: Reflex HPV testing is the recommended management for women with ASCUS Pap tests. Most studies show that the rate of HPV positivity decreases among women with increasing age, and that most young women with an ASCUS Pap test and a positive (+) HPV test harbor a low grade squamous intraepithelial lesion (SIL) rather than a high grade lesion. Therefore, it is expected that older women with an ASCUS Pap test and a positive (+) HPV result should have a higher incidence of high grade SIL detected on biopsy as compared to young women. Our aim was to study the biopsy outcome in patients with an ASCUS PAP test and a HPV reflex test stratified by age.

Design: The departmental computer system was searched for all women who had an ASCUS Pap test with a concurrent HPV reflex test. HPV reflex testing was performed on the residual Sure Path liquid vials using the Digene Hybrid Capture II method. Information on follow-up cervical biopsies was collected; only cases with a tissue biopsy were included. The follow-up data was stratified for age.

Results: Of 1349 ASCUS cases with a HPV reflex test, 705 were (+) for high-risk HPV. 64% of women younger than 30 years were HPV (+) versus 43% of women older than 30 years. 332 cases had tissue follow up; among these, 150 women were younger than 30 (range, 14 to 30 yrs); and 182 were older than 30 (range, 31 to 82 yrs).

Conclusions: 1. Women with an ASCUS Pap test younger than 30 years of age have a higher HPV positivity rate compared to those older than 30. 2. Most women with ASCUS who are HPV (+) harbor low grade SIL as opposed to high grade SIL regardless of age; however, the LSIL to HSIL ratio declines in women older than 50 years of age. 3. The incidence of high grade SIL in women with an HPV (+) ASCUS Pap test is similar across all age groups. 4. While the PPV remains the same, the NPV of HPV testing for detection of high grade SIL in follow up biopsies declines with age.

Histological Follow Up of ASCUS and HPV Reflex Testing Stratified by Age

Age	HPV	Benign	Low grade SIL	High grade SIL
≤30	+	16%	70%	13%
	-	25%	75%	0%
>30	+	21%	68%	11%
	-	39%	56%	5%
>50	+	26%	60%	14%
	-	43%	43%	14%

Positive (PPV) and Negative (NPV) Predictive Values of HPV Testing in Detection of High Grade SIL in Follow-up Biopsies of Women with ASCUS Stratified by Age

Age	PPV	NPV
≤30	13%	100%
>30	11%	95%
>50	14%	86%

380 ThinPrep® in Bile Duct Brushings: Analysis of Morphologic Parameters Associated with Malignancy and Interobserver Reliability

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Background: Recent work suggests the ThinPrep® method can improve diagnostic sensitivity and accuracy in bile duct brushings. However, the proportion of atypical and suspicious diagnoses remains high. The aim of this study was to identify the most useful morphologic features in ThinPrep® bile duct cytology.

Design: We evaluated 100 bile duct brushings prepared by ThinPrep®, all with either histology or long term clinical follow-up (55 malignant, 45 benign). Original cytologic diagnoses were: 25 benign, 25 atypical, 25 suspicious, 25 malignant. Morphologic parameters were evaluated by 3 experienced cytopathologists blind to clinical information and follow-up diagnoses. Parameters included cellularity, blood or diathesis, mitoses, inflammation, 3-dimensional groups, discohesive atypical cells, macronucleoli, and general malignancy features (nuclear membrane irregularity, chromatin clumping). The data was analyzed by intraclass correlation (ICC) and stepwise multiple logistic regression.

Results: Reviewers showed full disagreement in 6% of cases. The remaining showed either unanimous agreement (35%) or one degree of disagreement (59%). Sensitivity of the morphologic parameters varied from 2 to 62%; the highest sensitivity was for discohesive atypical cells, inflammation, and cellularity (62, 57, 47%). Specificity of the parameters varied from 20 to 100%; highest specificity was for mitotic figures and cellularity (100, 84%) and 3-dimensional clusters, macronucleoli, necrotic background (each 98%). Specificity for discohesive atypical cells was moderate (69%). ICC showed moderate to good reliability for cellularity, inflammation, 3-dimensional clusters, and discohesive atypical cells (0.70, 0.53, 0.45, 0.39). In multiple logistic regression analysis, only increased cellularity and overall nuclear features of malignancy separated benign from malignant. Both 3-dimensional clusters and macronucleoli predicted malignancy, but not at a level that reached statistical significance (p=0.149, 0.105) and thus were excluded from the model.

Conclusions: On ThinPrep® bile duct brushings, general nuclear features of malignancy and hypercellularity were most useful in distinguishing benign from malignant. Three-dimensional clusters and macronucleoli are specific but not sensitive for malignancy, and are not statistically significant in multivariate logistic regression models. Discohesive atypical cells showed only moderate sensitivity and specificity.

381 Squamous Atypia in Women with *Candida*, to "ASC" or Not?

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Background: Cervicovaginal candidiasis is a common infection frequently diagnosed in Pap tests. Candidiasis can result in cytologic alterations in squamous cells that may overlap with Bethesda System criteria for ASC-US and/or HPV infection. In this study, we correlate specific cytomorphologic features identified in patients with concomitant ASC-US and *Candida* to results of reflex high risk HPV DNA testing.

Design: During the calendar years 2004 and 2005, 4,136 Pap tests (5% of 83,971 total) were interpreted to contain *Candida*. Of these, 125 patients were concomitantly interpreted to have ASC-US (3%). Of these, 93 ThinPreps® (74%) underwent reflex HR HPV testing (hc2, Digene), representing the study set. Mean patient age was 31 years (91% premenopausal). Thirty-five percent of the patients had a prior history of cervical dysplasia. Slides were reviewed by two pathologists for the following parameters: intensity of inflammation, relative quantity of *Candida*, atypical parakeratosis (individual cells and groups), nuclear size >2.5 times an intermediate nucleus, chromatin character, squamous cell multinucleation, perinuclear ring, and vacuolated cytoplasm. Both pathologists also rendered a second opinion interpretation for each case. Findings from the slide review were compared to HPV testing in a blinded fashion.

Results: HPV DNA was identified in 29/93 (31%) of patients. Only 9/33 (27%) patients with a history of dysplasia were found to be HPV positive. Inference statistical analyses of HPV testing against all clinical and cytomorphologic parameters demonstrated an association with age, squamous cell multinucleation, and perinuclear ring (p values < 0.05). Both pathologists' second opinion interpretation of LGSIL and/or ASCUS correlated with positive HPV testing (p values < 0.0246). With a multivariate logistic regression model analysis of covariates with p values less than 0.25, only age and one of the pathologist's second opinions remained statistically significant.

Conclusions: Of the individual parameters evaluated, only age, squamous cell multinucleation, perinuclear ring, and pathologists' second opinions successfully correlated with positive HPV testing. A careful second slide review in cases with concomitant ASC-US and *Candida*, focusing on detection of perinuclear rings and squamous cell multinucleation may allow for more accurate classifications. Prior history of dysplasia may trigger "overcalls" of ASC-US in women with *Candida*.

382 Follow up of High Risk Human Papillomavirus (HPV) Tests with High Viral Loads: Correlation with Original Pap Test Interpretations and Biopsy Results

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Background: Testing for HPV using the Hybrid Capture II method (Digene) includes an assessment of the amount of HPV DNA present in the sample via a relative light unit (RLU) determination. To ascertain if cases showing high (>1000) RLU values show differing follow up compared to all HPV test results, we analyzed high RLU tests and compared the results to published data.

Design: All HPV tests performed from Surepath (TriPath) Pap test vials with RLU values greater than 1000 were identified. In each case, the original Pap test interpretation and all biopsy follow up data was recorded. The percentages of follow up as negative, CIN I, and CIN II+ were calculated. These figures were compared to reported follow up data to determine if high viral load cases performed in a different manner.

Results: 156 cases were identified for study. 11 cases (7%) had no follow up and were excluded, leaving 145 cases. The cytologic interpretations of the corresponding Paps were: Negative (NILM) - 3(2%), ASC-US - 87(60%), ASC-H - 5(3%), LSIL - 40(28%), and HSIL - 10(7%). Biopsy follow up compared to the original cytologic interpretation is showed in the table below.

Table 1. Results of the Original Cytology and Biopsy Follow up for the High Risk HPV DNA Cases

	Follow up Biopsy		
Original Cytology	Negative	CIN I	CIN II+
NILM	3 (100%)	0 (0%)	0 (0%)
ASC-US	28 (32%)	27 (31%)	15 (17%)
ASC-H	1 (20%)	2 (40%)	2 (40%)
LSIL	7 (18%)	25 (62%)	8 (20%)
HSIL	1 (1%)	2 (22%)	7 (70%)

Conclusions: In the ASCUS-LSIL Triage study (ALTS), all HPV+ ASC-US cases had a 26% chance of being CIN II+ on biopsy. In the present study, 17% of high RLU ASC-US cases were found to be CIN II+ on biopsy, which is very close to our own reported data (18%) previously. For ASC-H and HSIL cases, the present study showed that 40% and 70% of high RLU cases in these categories, respectively, had follow up of CIN II+, comparing very closely to ALTS and other studies. For LSIL cases, ALTS showed that 27% were CIN II+ on biopsy. In the present study, 20% of high RLU LSIL cases showed CINII+ on biopsy. This finding may represent a decline in CIN II+ as compared to ALTS, and may reflect a higher probability that high RLU cases are more likely to represent transient productive viral infections. Overall, however, the data from the study do not identify high RLU cases as significantly different from the overall HPV positive population, and therefore do not provide evidence for different management schemes.

383 Fine Needle Aspiration of Pediatric Head and Neck Masses: An Institutional Review

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Background: Fine needle aspiration biopsy (FNAB) is being increasingly used in the diagnosis and management of pediatric head and neck masses. We review our experience.

Design: FNABs of head and neck masses in patients with age ≤18 years were reviewed over a period of 3½ years (January 2003 to July 2006). Follow up information was obtained in all cases, including surgical and/or medical management. The surgical pathology diagnosis was reviewed for cases where subsequent histologic correlation was available.

Results: A total of 93 pediatric head and neck masses FNABs (mean age 10.4 years) from varying sites were performed in 37 males and 56 females. Eighty nine of the procedures (97%) were satisfactory for evaluation. FNABs were categorized as benign 79 cases (88%), malignant 7 cases (8%) and suspicious for malignancy 3 cases (4%). Malignant and suspicious for malignancy were combined as one category for statistical analysis. Definite histologic correlation was available on 25 (28%) cases. There was cyto-histologic concordance in 24 cases, with FNAB correctly identifying the specific histologic entity in 19 of 25 cases (76%), including the 10 cases classified as malignant / suspicious. Based on cyto-histologic correlation the sensitivity and specificity of the procedure was 90% and 100% respectively. The only one false negative case was related to sampling error. The benign results in the remainder 66 cases (72.5%) were considered reliable enough to allow either medical treatment or clinical follow up.

Conclusions: FNAB is a sensitive and specific method for diagnosis of pediatric head and neck masses. Based on our experience, pediatric head and neck FNAB results allow for definitive management protocol without the need for surgical biopsy for diagnostic purposes in vast majority of cases.

384 Persistent HPV Infection as Indicated by Repeatedly Positive HPV DNA Tests Is Predictive of the Presence of High Grade Cervical Squamous Intraepithelial Lesions (HSIL)

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Background: Detection of HSIL is imperative given their propensity for persistence or progression to invasive cancer. Since infection with high-risk human papillomavirus (HR HPV) is well known to be the primary cause of HSIL, identification of HR HPV (by HPV DNA tests) has been used to evaluate the risks for HSIL. Positive HPV tests are reportedly more common in HSILs than in normal cervix. Information, however, is scanty concerning the relationship between the persistence of HPV infection and the likelihood of detecting an HSIL. The aim of this study was to determine whether repeated HPV positivity, as compared with a transiently-positive HPV test, would be more predictive of the presence of an HSIL.

Design: Our archival file yielded 717 cases from the period 2003-2005, in which two HPV tests (Digene method) and at least one cervical biopsy were performed within a one-year time frame. HPV results were recorded as negative each time (HPV-), one time positive (HPV+1), or two times positive (HPV+2). Biopsy findings were divided into two categories: 1) normal or low grade squamous intraepithelial lesions (LSIL) and 2) HSIL. The negative and positive predictive values were calculated. Fisher Exact Test was used to compare the occurrence rates of HSIL between HPV- and HPV+1, and between HPV+1 and HPV+2 groups.

Results: Among 717 patients, 533 (74.3%) showed either normal cervix or LSIL, and 184 (25.7%) harbored HSIL. Of the 533 patients with normal cervix or LSIL, 84 (15.8%) were HPV-, 216 (40.5%) were HPV+1, and 233 (43.7%) were HPV+2. Among the 184 patients with HSIL, 5 (2.7%) were HPV-, 46 (25.0%) were HPV+1, and 133 (72.3%) were HPV+2. The negative predictive value of HPV- was 94.4%; the positive predictive value of HPV+1 was 17.6%; and the positive predictive value of HPV+2 was 36.3%. The occurrence rate of HSIL in the HPV+1 group was significantly higher than that in the HPV-1 group (p=0.0049), and the occurrence rate of HSIL was significantly higher in the HPV+2 group than in the HPV+1 group (p=0.0001).

Conclusions: Persistent HPV infection (manifested by 2 positive HPV DNA tests within one year), compared to transient HPV infection (shown by 1 positive HPV test in one year), is more predictive of the presence of an HSIL. Therefore, repeating an HPV DNA test within a 1 year time frame, regardless of the prior test result, may prove to be a more effective method of identifying HSIL.

385 Detection of HPV by ISH in FNA Biopsies of Cervical Metastases of Head and Neck Squamous Cell Carcinoma: A Tool for Identifying the Site of an Occult Primary

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Background: Many patients with head and neck squamous cell carcinomas (HNSCCs) present initially with a neck metastasis. In some of these cases the primary site is occult. Fine needle aspiration biopsy (FNA) is often the initial procedure for diagnostic evaluation of the lymph nodes. Therapeutic management and prognosis of the patients are dependent on whether or not the primary site of the tumors can be identified. We investigated the presence of HPV in FNA material of metastatic tumors, with known primary sites, by direct in situ hybridization (ISH). This was done to determine if HPV detection in lymph nodes can be useful in determining the origin of the metastatic tumors.

Design: Thirty FNA biopsies of metastatic cervical lymph nodes originating from HNSCCs (13 oropharynx, 13 oral cavity, and 4 larynx/hypopharynx) were recovered from our cytology files dating between 2002 and 2005. The aspirates were evaluated and grouped as non-keratinizing squamous cell carcinomas (NKSCCs) and keratinizing squamous cell carcinomas (KSCCs) based on the cytological features. ISH for high risk HPV, was performed on the alcohol fixed and Pap stained smears. The positive signals were visualized as black dot(s) inside the nuclei. For comparison, the corresponding excisional tissue was similarly analyzed for HPV in the formalin fixed and paraffin embedded sections.

Results: HPV was detected in 10/30 (33%) cases. Nine of the 10 HPV positive tumors (90%) had an oropharyngeal origin, and only one was from a non-oropharynx site (p = 0.001). Seven out of the 10 (70%) HPV positive aspirates were NKSCCs. The overall correlation of ISH results for HPV performed on cytological and histological material was 28/30 (93%).

Conclusions: ISH for HPV can be performed on alcohol fixed FNA material. HPV positive metastatic SCC in cervical lymph nodes strongly points to a primary site in the oropharynx. HPV detection in FNAs of metastatic cervical lymph nodes can be an effective tool in identifying the site of an occult primary.

386 Using Core Biopsy in Conjunction with Fine Needle Aspiration Decreases the Unsatisfactory Rate for Thyroid Biopsy

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Background: Fine needle aspiration (FNA) is the first line test to evaluate thyroid nodules and has both high sensitivity and specificity. However, the unsatisfactory rate is variable (range 5 to 43%) and is an important source of false negatives. Our institution has a low unsatisfactory rate (range 4%). The reasons include on-site evaluation for all cases and utilization of core biopsy (CB) (20 to 22 gauge) in conjunction with FNA. There is minimal literature on the use of thin CB for thyroid nodule(s) which we routinely use following 2 non diagnostic FNAs. We report our experience using data collected over the past calendar year.

Design: A retrospective search was performed in our pathology database for thyroid FNA/CB performed in IR and follow up surgical specimens during year 2005. The cytomorphology was reviewed for comparison with FNA. Cytologic-histologic correlation was performed on cases that underwent surgery. Records of all complications were retrieved from the IR data base.

Results: A total of 704 FNAs performed on 587 patients qualified for the study with 92 (15.7%) males and 495 (84.3%) females. The mean age was 51.4 years (range 18 to 87). Among the 704 FNAs, 214 had FNA in conjunction with CB (30.4%), and 490 (69.6%) had FNA alone. The mean number of passes was 1.97 for the 704 FNAs, 2.13 for FNA in conjunction with CB, and 1.9 for FNA alone. The FNA diagnoses for the 704 cases were: unsatisfactory 19 (2.7%), negative 473 (67.2%), indeterminate for neoplasm 135 (19.2%), neoplasm and suspicious for malignancy 48 (6.8%), and malignancy 29 (4.1%). A total of 139 FNAs (131 patients) had histologic follow-up, including 41 papillary carcinomas, 1 follicular carcinoma, 42 follicular adenoma, and 55 adenomatoid nodules. There was one possible false positive case (false positive rate 1.0%) and two false negative cases due to sampling error (false negative rate 4.8%). Among the 214 cases with CB, there were no major complications, and only two patients had local rash and itching. The morphology prepared from the thin CB is more cellular and shows larger "tissue fragments" compared to FNA.

Conclusions: FNA in conjunction with a thin CB can significantly reduce the unsatisfactory rate of thyroid FNA, especially in firm, chronically inflamed or fibrotic nodules and in vascular lesions that yield bloody specimens. In experienced hands, thyroid CB is a very safe technique with no more complications or discomfort than thyroid FNA.

387 Fine Needle Aspiration Biopsy of Proliferative Breast Lesions in Patients with Palpable Mammary Masses. A Review of 172 Cases and Correlation with Histologic Diagnosis

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Background: The diagnosis of proliferative breast lesion (PBL) and PBL with atypia (APBL) continues to be an area of debate in breast FNA cytology. Sampling error and heterogeneity of breast lesions complicates the histologic correlation of FNA result further.

Design: A computer-based search of the files of the Department of Pathology, LAC+USC Medical Center was carried out to retrieve breast FNAs performed from 2000 to 2005 which were diagnosed as PBL. Both cytological and surgical slides of these cases were reexamined. A cytological diagnosis of PBL or APBL was used if the findings of the proliferative breast lesion did not fit a more specific category, such as carcinoma, fibroadenoma (FA), or FCC.

Results: 3,934 breast FNAs were performed on palpable breast masses during the perioid. 317 (8.1%) were cytologically diagnosed as PBL with or without atypia. 201 cases (63.4%) had subsequent surgical biopsies. After the cytologic smears were reviewed, 10 cases were diagnosed as FA, 6 as carcinoma, 12 as suspicious for carcinoma, and 1 as unsatisfactory; these 29 cases were excluded from this study. Table 1.

Conclusions: APBL was clinically significant because it was associated with a significant increased likelihood of malignancy compared to PBL without atypia. Most of these malignancies showed hypocellularity and low nuclear grade in the FNA smears. The term atypiain breast cytology denotes uncertainty and increased risk and is not equivalent to the term atypical as ADH used in histology. Most FAs showed some degree of UDH in surgical biopsies, and areas of UDH likely were reflected in most preceding FNA findings of PBL. Most cases in this series lacked one or all major cytologic features of FAs.

Correlation of cytologic and histologic diagnosis in 172 proliferative breast lesions

Histology	No. of cases	APBL	PBL
Malignant	21 (12%)	19 (37%)	2 (2%)
Inv ductal ca	8 (5%)	6 (12%)	2 (2%)
Inv lobular ca	5 (3%)	5 (10%)	0
DCIS	5 (3%)	5 (10%)	0
Other malignant	3 (2%)	3 (6%)	0
Benign	151 (88%)	33 (63%)	118 (98%)
FA	99 (57%)	16 (31%)	83 (69%)
FCC/UDH	12 (7%)	0	12 (10%)
Papilloma	8 (5%)	4 (8%)	4 (3%)
Adenomyoepithelioma	5 (3%)	0	5 (4%)
FCC with UDH	5 (3%)	0	5 (4%)
ADH	3 (2%)	2 (4%)	1 (1%)
ALH	2 (1%)	1 (2%)	1 (1%)
Atypical papilloma	3 (2%)	3 (6%)	0
PT , benign	2 (1%)	0	2 (2%)
Other benign	12 (7%)	7 (11%)	5 (4%)
Total	172	52 (30%)	120 (70%)

388 Clinical Significance of Atypical Glandular Cells in Conventional Smears: A Histologic Follow-Up Study from a Large County Hospital

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Background: This atypical glandular cell (AGC) category remains a diagnostic challenge to both clinicians and cytopathologists. The aim of this study was to determine the rate of AGC and the incidence of clinically significant lesions on subsequent histologic follow-up among a patient population that consists predominantly of low-income and minority women.

Design: Conventional pap smears diagnosed as atypical glandular cells of endocervical origin (AGC-EC), atypical glandular cells of endometrial origin (AGC-EM), and atypical glandular cells not otherwise specified (AGC-NOS) from 2003 to 2005 at the LAC+USC Medical Center were retrieved from the department files. The cases were divided into the following diagnostic categories: ASCUS & AGC, AGC-EM, AGC-EC and AGC-NOS. The histologic diagnoses were correlated with the cytologic diagnoses.

Results: In 64,378 conventional cervicovaginal smears examined during the 3-year study period, AGC was reported in 525 (0.80%) cases, with follow up surgical specimens in 460 (87.6%) of these cases including 38 cone/leep biopsies and 90 hysterectomies. The cyto-histo correlation of these 460 cases is listed in Table 1.

Table 1. Correlation of the AGC Cases with Premalignant or Malignant Lesions in Tissue Biopsies

	AGC & ASCUS	AGC-EM	AGC-NOS	AGC-EC	Total
No. of cases	68 (14.8%)	36 (7.8%)	187 (40.7%)	169 (36.7%)	460
Mean ages	44	50	45	41	44
Sqamous cell lesions	9 (13.2%)	1 (2.8%)	10 (5.3%)	13 (7.7%)	33 (7.2%)
Cervical glandular lesions	0	0	5 (2.7%)	14 (8.3%)	19 (4.1%)
Endometrial lesions	3 (4.4%)	21 (58.3%)	38 (20.3%)	3 (1.8%)	65 (14.1%)
Ovarian lesions	0	1 (2.8%)	4 (2.1%)	1 (0.6%)	6 (1.3%)
Total	12 (17.6%)	23 (63.9%)	56 (29.9%)	30 (17.8%)	121 (26.3%)

Conclusions: In our study population, 26.3% cases with AGC had cancerous or dysplastic squamous or glandular lesions of the exocervix, endocervix, endometrium or ovary, with the most common origin being endometrium. A diagnosis of AGC-EM is the most clinically significant with the highest percentage (63.9%) of women showing premalignant and malignant lesions on subsequent histology. Patients with AGC on Pap smears should undergo immediate intensive diagnostic studies, including colposcopically directed biopsy with endocervical curettage to detect cervical lesions, and endometrial curettage and biopsy to detect endometrial lesions.

Dermatopathology

389 Evaluation of CD10 Expression in Spindle Cell Lesions of the Skin with Emphasis on Atypical Fibroxanthoma

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Background: CD10, a cell surface endopeptidase present in a number of normal cells, carcinomas and sarcomas, has been reported to be a sensitive marker for atypical fibroxanthoma (AFX). However, its expression in various cutaneous spindle cell lesions has not been studied. We evaluated the expression, sensitivity and specificity of CD10 in AFX, in comparison with various spindle cell tumors that may be in its differential diagnosis.

Design: CD10 immunohistochemistry was performed on representative paraffin-embedded sections of 17 AFX, 12 dermatofibrosarcoma protuberans (DFSP), 10 cellular dermatofibromas (CDF), 2 epithelioid dermatofibromas (EDF), 12 spindle cell carcinomas (SCC), 9 spindle cell melanomas (SCM), 7 leiomyosarcomas (LMS) and 2 fibrosarcomas with myofibroblastic differentiation (FMD). Additional 11 malignant fibrous histiocytomas (MFH) were stained for comparison. Diagnoses in all cases were based on morphology, clinical data and immunohistochemistry. Cases were analyzed for pattern of CD10 expression in tumor cells and stroma.

Results: All AFX cases (100%) showed strong and diffuse membranous positivity for CD10 in spindle cells and majority of pleomorphic giant cells (PGC). The latter showed bright membranous and weak cytoplasmic staining. An identical pattern was observed in 10 (100%) CDF (including 4 with monster cells), 8 (73%) MFH, and 2 (100%) FMD cases. The 2 remaining MFH had focal and weak staining only in PGC. Two positive MFH cases contained numerous osteoclastic-like GC, which did not stain for CD10. All (100%) LMS (including 6 that contained PGC), 8 (89%) SCM, and 2 (100%) EDF were negative. One (11%) SCM showed patchy, strong membranous and cytoplasmic staining. CD10 was negative in tumor cells of 11 DFSP (92%), but the surrounding fibroblastic reaction was strongly positive. One case (8%) had diffuse, strong membranous staining. In SCC, 8 cases (67%) were negative and 4 (33%) showed patchy staining of variable intensity within both spindle and PGC.

Conclusions: 1. The sensitivity and specificity of CD10 in AFX were 100% and 67%, respectively. 2. Strong and diffuse CD10 expression was seen within spindle cells and PGC in AFX, CDF and MFH. 3. All LMS, and most DFSP and SCM did not express CD10. However, special care must be taken, since the periphery of some of these tumors may be positive, presumably from reactive fibroblasts around tumor cells. 4. CD10 expression in SCC is variable, possibly because of mixed cell population. Positivity in PGC was observed in a minority of cases.

390 The Evaluation of T Regulatory Foxp3 Expression in Cutaneous T-Cell Lymphocytic Infiltrates

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Background: A growing body of literature exists regarding the role of thymically derived CD4 regulatory T cells (Tregs). The primary function of Tregs is to maintain immunologic tolerance by suppressing self-reactive T cells that have escaped negative selection in the thymus. The development of Tregs depends heavily on the transcription factor Foxp3--the lack of which results in fatal autoimmune lymphoproliferative disease. The role of Tregs in T cell lymphoma has been investigated with conflicting results. This study evaluates the expression and distribution of Foxp3 in various reactive and neoplastic cutaneous T cell disorders.

Design: A variety of T cell lymphocytic infiltrates cases were selected prospectively from both routine and consultative dermatopathology practice and categorized into one of three groups: (1) reactive lymphomatoid; (2) endogenous pre-lymphomatous T cell dyscrasia; and (3) T cell lymphoma. Foxp3 expression was evaluated by standard immunohistochemistry. TCR-beta gene rearrangement studies were performed using multiplex PCR.

Results: Of the 76 cases, 32 (42%) were classified as reactive lymphomatoid, 27 (36%) as T cell dyscrasia, and 17 (22%) as T cell lymphoma. The reactive lymphomatoid category included cases demonstrating lymphoid atypia with potential clonality, but were restricted to those resolving with removal of an identifiable antigenic trigger. The dyscrasia category was represented by pityriasis lichenoides chronica, pigmented purpuric dermatosis, alopecia mucinosa, and large plaque parapsoriasis. The mean Fox3p positivity was 17% overall--22% for the reactive lymphomatoid cases, 15% for the dyscrasias, and 11% for the lymphomas. The most aggressive lymphomas tended to have rare Fox3p cells (<5%), including two subcutaneous panniculitis-like T cell lymphomas, two CD4 NK-like T cell lymphomas, a primary cutaneous CD8 cytotoxic T cell lymphoma and a tumor-stage mycosis fungoides. TCR-beta gene rearrangements studies were available for 66 cases. Of these, monoclonality was seen in 18 (27%), oligoclonality in 20 (30%), and polyclonality in 28 (43%). The mean Fox3p positivity was 13% for the monoclonal cases, 17% for the oligoclonal cases, and 20% for the polyclonal cases.

Conclusions: Foxp3+ T regulatory cells may play a role in controlling the extent of clonal T cell proliferations in the skin with a lack of T regulatory cell function permissive to clonal expansion.

391 Cutaneous Neoplasms with Pagetoid Cells: An Immunohistochemical Study of Mammaglobin Expression

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Background: Mammaglobin belongs to the secretoglobin family of small epithelial secretory proteins and has been characterized as a fairly specific marker for breast carcinoma when employing molecular techniques. Reports on the use of mammaglobin immunohistochemistry are fewer but have shown positive results in up to 80% of cases of breast carcinoma. Neoplasms with pagetoid cells include mammary Paget's disease (MP), extramammary Paget's disease (EMP), melanoma in situ (MIS), and Bowenoid squamous cell carcinoma (BSCC). Our aim was to investigate mammaglobin expression in these lesions, and characterize its utility as an immunohistochemical marker in MP and EMP.

Design: We studied mammaglobin expression using a monoclonal antibody in the following cases: 12 EMP, 5 MP, 5 MIS, and 5 BSCC. All cases of MP were associated with either history of or concurrent breast carcinoma in women.