Results:

Subendothelial Layer Thickness

Case	RA mm	LA mm	LV mm	PV mm	EM LA μ	EM RAμ
Adult n=7	0.3	0.9*	0.04	0.5*	0.0512*	0.0348*
Adol n=4	0.2	0.5	-	-	0.0435*	0.0409
Infant n=4	0.2	0.2	-	-	0.0432*	0.0374

*Significant differences are noted between average intimal thicknesses in the adult LA and pulmonary vein (PV) and in collagen fibril diameters, respectively, in the adult LA and RA; adolescent (Adol) LA and adult LA, and the infant LA and adult LA; $p<0.001$.

Conclusions: The subendocardial thickness is greatest in the adult left atrium and increases with age; however, the right atrial intimal thickness does not. In adults, left atrial collagen fibers are thicker than right atrial collagen fibers and are approximately the same diameter in the pediatric age group. Adult left atrial collagen fibers are thicker than those of pediatric left atria and adult right atrial collagen fiber thicknesses are similar to those of the pediatric age group. The increased thickness of the adult left atrial subendocardial layer may represent a "cardiac endoskeleton" in response to shear stresses and correlate with increased stiffness of the left atrium with age.

297 Endomyocardial Biopsy Guided by Electroanatomic Voltage Map in Arrhythmogenic Right Ventricular Cardiomyopathy (ARVC): Histologic and Histomorphometric Findings

PF Silenzi, A Avella, A Pappalardo, C Tondo, G d'Amati. Sapienza University, Rome, Italy; S. Camillo-Forlanini Hospital, Rome, Italy.

Background: Endomyocardial biopsy (EMB) is used to show RV fibrofatty replacement in ARVC, but its sensitivity is low. Recent data support CARTO system as a new approach to identify low-voltage regions of fibrofatty myocardial replacement.

Design: To improve EMB accuracy for ARVC diagnosis, we hypothesized a RV sampling focused on pathological areas identified by voltage mapping. Twenty-two consecutive patients (10 M,12 F; mean age: 34 ± 10 years) were divided in two groups: Group A with evidence of ARVC (fulfilment of standardised noninvasive diagnostic criteria: 11 pts); Group B with suspicion for ARVC (ventricular arrhythmias, with LBBB morphology, but inadequate number of ARVC Task Force diagnostic criteria: 11 pts). An electroanatomic (EA) reconstruction of RV was performed in all pts. In patients with RV pathological segments (all pts from group A, three refused consent for EMB, and 8 Group B pts) an EMB focused on low-voltage areas was attempted. A disposable bioptome was inserted in the RV and positioned, under fluoroscopic guidance, as close as possible to the catheter tip targeting low-voltage areasUp to 6 specimens were obtained from low-voltage regions. Multiple histologic sections (15-30) were obtained from each sample and stained with H&E, Masson Trichrome and antibodies for CD3, CD20 and CD68. Computerized histomorphometry was also performed on all trichrome-stained sections, to measure the extent of fibro-fatty replacement

Results: Biopsies focused on low-voltage RV segments revealed myocardial fibrofatty replacement consistent with a histologic diagnosis of ARVC in 6/8 Group A patients (75%) and in 7/8 Group B patients (87%). Histomorphometric analysis documented a 27 ± 17% mean amount of fibrofatty myocardial substitution, with no significant differences between group A and group B. Also, in the whole study population there was no significant difference in the amount of fibrofatty replacement in the different biopsy sites (RV efflux: 22%; inferior wall: 30.5%; anterior wall: 33%; apex: 29%).

Conclusions: Our results show that: a) EMB targeting low-amplitude areas is a safe and highly sensitive technique for ARVC diagnosis confirmation; b) there is a high prevalence of RV low-voltage segments also in patients with clinical suspicion for ARVC; c) the amount of fibrofatty replacement in this selected population is similar between patients with clinical evidence and suspicion of ARVC, and does not vary in the different biopsy sites.

298 Pathology of Viridans Streptococcal Endocarditis Revisited

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Background: The viridans group of Streptococci accounts for 40-60% of community-acquired subacute infective endocarditis of native heart valves. These are slow-growing bacteria that produce glycocalyx (exopolysaccharides). The glycocalyx can induce platelet aggregation important in the formation of vegetations and appears to protect the bacteria from host immune defense mechanisms and from the action of antibiotics.

Design: From July, 2005 to June, 2007, 83 cases of infective endocarditis were retrieved from the surgical pathology files. Eighteen cases were confirmed to be caused by viridans streptococci by history of positive blood cultures. The histologic features of streptococcal endocarditis were reviewed. Special staining with Gram and periodic acid Schiff (PAS) were routinely performed. Fluorescent in situ hybridization (FISH) using a FITC-labeled genus-specific 16S rRNA probe for Streptococcus spp. was performed on 5 of the 18 cases and 5 negative controls.

Results: Streptococcal endocarditis involved 11 native and 7 bioprosthetic valves. The aortic valve alone was affected in 10 cases, mitral valve alone in 1 case, aortic and mitral valves in 5 cases and mitral and tricuspid valves in 2 cases. The vegetations showed Gram-positive cocci that were also intensely stained with PAS. The following histologic features on H&E were consistently observed: 1. exuberant fibrin-rich vegetations with evidence of granulation tissue; 2. readily identifiable pale foamy macrophages which contained microorganisms that stained PAS-positive and Gram-positive; and 3. presence of multinucleated giant cells in the vegetations but not in the valve stroma. Tissue cultures were obtained at the time of excision in 13 cases and were negative in 9. FISH demonstrated intense staining of clusters of cocci in the vegetations and faint staining of intracellular microorganisms.

Conclusions: Viridans streptococcal endocarditis shows distinct histologic features that include pale foam cells identifiable on H&E, Gram and PAS-positive cocci and intracellular organisms in macrophages and giant cells. The production of glycocalyx by viridans streptococci accounts for the tinctorial properties that react with the Schiff reagent. The presence of pale foamy macrophages and giant cells in vegetations should prompt pathologists to perform PAS staining which is very helpful in the diagnosis of viridans streptococcal endocarditis. Molecular detection of these organisms can confirm the diagnosis of infection by viridans streptococcus in culture-negative endocarditis.

299 Patterns of C4d and C3d Immunofluorescence Staining in the Evaluation of Antibody-Mediated Rejection in Heart Transplants

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Background: The diagnosis of acute antibody-mediated rejection (AMR) in cardiac transplantation is based on a set of clinical, serologic and histopathologic findings. The immunopathological evaluation of endomyocardial biopsies for AMR needs further standardization and correlation with clinical findings.

Design: Frozen sections of all heart transplant endomyocardial biopsies (EMBs) from October 2006 to August 2007 were routinely evaluated for AMR with C4d and C3d immunofluorescence staining. Biopsies were scored semi-quantitatively from 0 to 4+. Clinical information was obtained by a retrospective review of electronic medical records.

Results: A total of 1223 consecutive EMBs from 316 adult heart transplant patients were evaluated. The number of EMBs per patient ranged from 1 to 23. Fifty one out of 316 patients showed evidence of complement deposition in at least one occasion. Based on the presence of complement deposits, patients were divided into 4 groups depending on the pattern of immunofluorescence staining. Group A (n=15) had diffuse linear capillary staining of 2+ or higher with C4d and C3d. Group B (n=15) had diffuse linear capillary staining of C4d only. Group C (n=11) had focal capillary staining of C4d only. Group D (n=10) had linear perimyocytic staining of C4d with or without C3d. Hemodynamic compromise (decreased cardiac output/index, decreased ejection fraction, rise in pulmonary capillary wedge pressure) was noted in 87% (13/15) of group A patients, 6.7% (1/15) of group B patients and none in groups C and D. There were 6 deaths that occurred, 5 group A patients died of cardiac-related causes and 1 group D patient died of sepsis.

Conclusions: The majority of patients with concurrent C4d and C3d capillary deposition had cardiac allograft dysfunction. C4d capillary staining alone does not discriminate between those who have hemodynamic compromise and those who are asymptomatic. Focal and perimyocytic complement deposition can be found in asymptomatic patients. Long-term follow-up is needed to correlate immunofluorescence staining pattern with overall prognosis in heart transplant recipients.

300 Spontaneous Angiogenesis in Human Ischemic Hearts Is Not Clearly Correlated with VEGF Gene Expression

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Background: VEGF is a critical growth factor for angiogenesis. However, relationship between VEGF level and endothelial proliferative activity in end-stage human heart is not well-established.

Design: We have studied 11 human hearts explanted at the time of transplantation which displayed severe coronary artery disease (ischemic hearts). As controls, we also studied 8 explanted hearts from patients with idiopathic dilated cardiomyopathy. Endothelial proliferative indices were measured using a double immunostaining method for PCNA and Ulex Europaeus lectin. Proliferating endothelial cells were detected in capillaries and in a few arteries.

Results: The endothelial proliferative indices were low in both groups of hearts, but the proliferation was significantly elevated in the ischemic hearts compared to the cardiomyopathic hearts (mean ± SD = 0.12 ± 0.16% vs. 0.009% ± 0.009%, respectively). Within the ischemic hearts, there was also an increased endothelial proliferative index in the region of scars (myocardium = 0.12 ± 0.16%, myocardium adjacent to scar = 1.41 ± 0.88%, scar tissue = 1.45 ± 1.34%). Immunostaining for VEGF protein revealed no detectable VEGF in the myocardium, although focal VEGF positivity was seen in the atherosclerotic plaques of the ischemic hearts. Based on ELISA determinations, low-to-absent levels of VEGF protein were also seen in myocardial samples from both groups without significant differences. Semi-quantitative RT-PCR detected VEGF receptor flk-1 mRNA expression in ischemic heart myocardium, but not in cardiomyopathic myocardium.

Conclusions: These data suggest that the low but elevated level of endothelial proliferative activity is spontaneously present in end-stage ischemic human hearts and is not clearly associated with VEGF expression. However the significant expression of flk-1 in these ischemic hearts is consistent with these tissues being possibly responsive to administered VEGF.

Cytopathology

301 Imuncytochemical Study of the Urine Cytological Preparations of the Secondary Prostatic Adenocarcinoma of the Urinary Bladder

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Background: Involvement of the urinary bladder by prostatic adenocarcinoma (PAC) occasionally occurs and is usually associated with high grade and high stage PAC. In this study, we analyzed urine cytological findings in patients with secondary involvement of the urinary bladder by PAC with the help of the immunocytochemistry.

Design: Urine specimens from 15 patients with history of PAC with suspected secondary involvement of the urinary bladder and adequate urine cytospin specimens were included in the study group. The cases were divided into two groups: 1) prospective study group: 3 cases and 2) retrospective study group: 12 cases which were retrieved from the Cytopathological files. The urine cytology specimens (cytospins) from all the cases in the study group were submitted for PSA immunocytochemistry. Additional immunostaining

for cytokeratin 7 (CK7) was performed if PSA immunoreactivity was negative.
Results: All cytospin smears showed atypical cells characterized by large round and uniform nuclei with prominent nucleoli and dense cytoplasm. They were present as single cells or in cell groups simulating urothelial carcinoma. The diagnosis of PAC was made if the atypical cells were either immunoreactive for PSA or nonreactive for CK7. The urothelial cells were either PSA- or CK7+. The immunostaning supported the PAC diagnosis in all 3 cases in the prospective and 2 cases in the retrospective groups. The remaining 10 cases in the retrospective group were diagnosed as negative: 3, atypia: 5 urothelial carcinoma: 2. The positive diagnosis for PAC was based on the PSA immunoreactivity or nonreactivity to CK7 and the cytological atypia.
Conclusions: Immunostaining for PSA and CK7 performed on cytospins of urine specimens of patients with prior history of high grade and/or stage of PAC is helpful to make the positive diagnosis of bladder involvement of PAC.

302 Determination of Proliferative Rate in Pancreatic Endocrine Tumors on Endoscopic Fine Needle Aspiration Material: Correlation with Behavior

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Background: Pancreatic endocrine tumors (PETs) are slow growing neoplasms with distinct morphologic characteristics that behave less aggressively than adenocarcinomas. The malignant potential of these neoplasms is difficult to predict. The goal of this study was to evaluate the role of endoscopic ultrasonography-guided fine needle aspiration (EUS-FNA) in the preoperative diagnosis of PETs and investigate whether the proliferation (Ki-67) index determined on this cytologic material could help predict their behavior.
Design: Seventeen EUS-FNAs of PETs performed from 2003 to 2007 were evaluated retrospectively. The study group comprised 11 males and 6 females (mean age 62.2 years; range 31-77, male to female ratio 1.8:1). Diff-Quick and Papanicolau stained smears were reviewed, and immunocytochemical stains were performed for chromogranin A, synaptophysin, and Ki-67 on cell block sections. Ki-67 index was evaluated using ACIS ChromaVision Automated Assisted Image Analysis software. Clinical follow-up (mean 20.4 months; range 2-43) was compared with Ki-67 index.
Results: The overall survival was 88%. Nine of 17 (52.9%) patients developed lymph node and/or hematogenous metastases. Ki-67 index in PETs with no metastases was lower (mean 6.3; range 2-13) than in clinically aggressive tumors (mean 17.5; range 3-54), p=.0002. Whereas the tumors with no metastatic spread had low Ki-67 index (all below 13, median 6.5), clinically aggressive tumors showed a wider range of Ki-67 index (up to 54, median 5.0). Both patients that died of disease had a Ki-67 index of 4.
Conclusions: Proliferation rate in PETs can be determined using image analysis on cytologic material. Although tumors with metastatic potential tend to exhibit a higher Ki-67 index, there is significant overlap with more indolent neoplasms. Our findings suggest that a Ki-67 index cut-off of 2% as recommended by the WHO does not accurately predict malignant behavior of PETs; however, all tumors with Ki-67 index above 13 developed metastases.

303 Expression of CD25 in B Cell Lymphoproliferative Disorders

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Background: Interleukin 2 (IL-2) plays a major role in T cell activation and cellular immune responses. Recent murine data finds subsets of normal B-cells also express the alpha subunit of the IL-2 receptor, CD25, although apparently with low frequency in lymph nodes. CD25 positivity is part of the diagnostic immunoprofile of T-cell lymphoproliferative disorders such as adult T cell leukemia/lymphoma and anaplastic large cell lymphoma. Some B-cell types, such as hairy cell leukemia and Hodgkin/Reed Sternberg cells are also known to express CD25, but since it is rarely included in primary immunophenotyping panels the frequency of its expression in human B-cell lymphoma is not well studied. Clinical trials have examined anti-CD25 in treating adult T cell leukemia/lymphoma, with promising results. The aim of the present study was to examine the expression of CD25 in a variety of B-cell lymphoproliferative disorders in order to determine if some of B-cell lymphomas might be potential candidates for anti-CD25 therapy.
Design: In our cytology laboratory lymphoid samples are assessed for CD25 in combination with FMC7 (DAKO). Immunophenotyic data obtained by laser scanning cytometry was reviewed to obtain a series of 100 consecutive B cell lymphomas (93 fine needle aspirates, 7 body fluids). Using original flow cytometry standard (fcs) files, CD25 vs FMC7 histograms were re-analyzed and the percentage of B-cells showing expression of CD25 was calculated.
Results: Cases examined included follicular lymphoma (41), large B cell lymphoma (14), mantle cell lymphoma (6), marginal zone lymphoma (11), small lymphocytic lymphoma (12), post transplant lymphoproliferative disorder (2), lymphoplasmocytic lymphoma (2), hairy cell leukemia (1) and unclassified B cell lymphoma (11). 71 cases of B-cell lymphoma were completely negative for CD25. 23 cases showed dim co-expression of CD25+/FMC7+ in less than 8% of cells. Two cases of mantle cell lymphoma showed co-expression of CD25+/FMC7+ on 21 and 69 percent of cells and two large B cell lymphomas (CD19+ CD20+) showed moderate expression of CD25 but were FMC7 dim or negative. A single case of diffuse large B cell lymphoma was moderatly co-expressing both markers in 47 percent. The single case of hairy cell leukemia showed bright CD25 in all cells.
Conclusions: The majority of B cell lymphomas are negative, or only dimly positive for surface CD25. However a few show moderate expression in a majority of the cells and might be potential candidates for incorporation of anti-CD25 treatment in a chemotherapeutic regimen.

304 Women ≥50 with ASC-US Have a Lower Rate of HPV Infection and Are More Likely To Be Infected with Lower Risk and Uncommon HPV Genotypes Compared to Women <50

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Background: HPV DNA reflex testing is currently used in most institutions to triage women with a diagnosis of ASC-US for colposcopy. Studies have shown that the rate of HPV infection and the rate of underlying dysplasia decline with age in women with ASC-US. The aim of this study was to assess if the lower rate of dysplasia following a diagnosis of ASC-US in women over 50 is due solely to the lower rate of underlying HPV infection or if a change in the relative proportion of HPV genotypes is a contributing factor.
Design: We identified cases with HPV DNA tests performed on Surepath(r) liquid-based Papanicolaou Tests (LBPT),) between 04/01/01 and 08/31/06 in a large multi-hospital health care system serving a predominantly suburban population. HPV DNA tests were performed by a PCR-based method allowing genotyping. The women's age, LBPT diagnosis, presence and type of HPV and follow-up biopsy diagnoses made within 6 month were entered into a spreadsheet and statistical analysis was performed with SPSS. HPV types included in the high-risk Digene hybrid capture test were classified as high-risk; all other types as low risk. HPV types were also assigned to clades (a1/8/10, a3/4/15, a5/6, a7, a9/11, a14).
Results: Among the 9016 women with a diagnosis of atypical squamous cells of undetermined significance (80% ASC-US and 20% ASCUS) who had HPV DNA tests performed during this interval there were 1271 (14.1%) women ≥50 (mean age 57.3, median 55) and 7745 women <50 (mean age 31, median 30). HPV testing showed that 226 (18%) of women ≥50 were positive for any HPV type as compared to 2873 (37.1%) in women <50. As a percentage of all HPV+ cases, hr-HPV types were less common (26 vs 49%, p<0.001) while lr-HPV types were more common (64 vs 45%, p<0.001) as were untypeable HPV (10 vs. 6%). HPV types belonging to clade A9/11 including HPV16 were significantly less common in women ≥50 (OR= .44 (.3, .6), p<0.001), as were those belonging to clade A7 including HPV 18 (OR=.6(.4, .9), p=.03). In contrast, HPV types belonging to clade A3A4A15 (OR=3 (2.1, 4.2), p<.001) were significantly more common in women≥50. Follow-up biopsies were available in 16% of women ≥50 and 23% of women <50. CIN2/3 was diagnosed in 4% of women ≥50 and 14% of women <50 biopsied.
Conclusions: Women ≥50 with ASC-US have a significantly lower rate of HPV infection and a shift towards low-risk and more uncommon HPV genotypes. These factors contribute to the lower rate of CIN2/3 follow-up diagnoses.

305 Should Reflex hrHPV DNA Detection Be Performed in Women with ThinPrep Tests without TZ/ECS?

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Background: Controversy continues regarding the relation between the presence of transformation zone and endocervical cells (TZ/ECS) and the finding of abnormalities in ThinPrep Pap tests (TPPT), even though sampling of the TZ/ECS has been regarded as a quality indicator in cervical screening.
Design: Data from 143438 TPPT obtained over an 18 month period from Magee-Womens Hospital were analyzed to asses the relation between the prevalence of squamous intraepithelial lesions and the presence of TZ/ECS. Chi-Square test was performed using SAS 9.1.
Results: Both LSIL and HSIL prevalences were significant higher in women with TPPT with TZ/ECS than that without TZ/ECS among women aged less than 50 ($p=0.9x10^{-3}$, and $p<1x10^{-4}$). Detailed results were shown in table 1 and 2.

TABLE 1

		TZ present			TZ absent		
Age	Total	LSIL (%)	95% CI	Total	LSIL (%)	95% CI	P
10-	5954	849(14.3)	13.4-15.2	1239	129(10.4)	8.7-12.1	3x10-4
20-	30048	2665(8.9)	8.6-9.2	5858	490 (8.4)	7.7-9.1	0.212
30-	25014	811 (3.2)	3.0-3.5	4614	108 (2.3)	1.9-2.8	0.001
40-	26546	577 (2.2)	2.0-2.4	4870	95 (2.0)	1.6-2.3	0.323
50-	20211	256 (1.3)	1.1-1.4	6554	77 (1.2)	0.9-1.4	0.560
60-	8306	75 (0.9)	0.7-1.1	4224	23 (0.6)	0.4-0.8	0.031
All	116079	5233(4.5)	4.4-4.6	27359	922(3.4)	3.2-3.6	<1x10-4

Table 2

		TZ present			TZ absent		
Age	Total	HSIL (%)	95% CI	Total	HSIL (%)	95% CI	P
10-	5954	82 (1.38)	1.08-1.68	1239	8 (0.65)	0.20-1.10	0.035
20-	30048	429 (1.43)	1.30-1.56	5858	32 (0.55)	0.36-0.74	<1x10-4
30-	25014	199 (0.80)	0.69-0.91	4614	19 (0.41)	0.18-0.61	0.005
40-	26546	79 (0.30)	0.23-0.37	4870	7 (0.14)	0.04-0.25	0.059
50-	20211	36 (0.18)	0.12-0.24	6554	8 (0.12)	0.04-0.20	0.330
60-	8306	12 (0.15)	0.06-0.22	4224	5 (0.12)	0.02-0.22	0.708
All	116079	837 (0.72)	0.67-0.77	27359	79 (0.29)	0.23-0.35	<1x10-4

Conclusions: 1. The presence of TZ/ECS can affect the detection of SIL lesions in women younger than 50 years. 2. Recently we reported that HC2 hrHPV DNA detection was independent of cytologic sampling of TZ/ECS. 3. We recommend that reflex hrHPV DNA testing in TPPT without TZ/ECS should be performed routinely.

306 Clinical Significance of 9p21 Loss Detected by Urovysion FISH Analysis of Urine Samples

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Background: UroVysion (Abbott/Vysis, Downer's Grove, IL) FISH analysis is used in patients (pts) under surveillance for recurrent urothelial carcinoma (UC) and in pts presenting with hematuria. A FISH + result is defined as chromosomal gain of ≥2 chromosomes (3,7 or17) in ≥4 cells, homozygous deletion of 9p21 in ≥12 cells, or

isolated gains of chromosomes 3,7,or 17 in ≥10% of cells. There are limited follow up data in the literature for the subset of pts with FISH+ by 9p21 loss.
Design: We studied consecutive FISH + pts with homozygous deletion of 9p21 over a 2yr period from 2004-2006. All pts had ≥12mos available follow up. Pts were stratified by indication for FISH testing; either hematuria and no history of UC or UC surveillance. The UC surveillance pts were compared to an age matched FISH negative control group.UroVysion FISH testing was performed on ThinPrep slides (Cytyc Corporation, Marlborough, MA). All available cystoscopy, cytology and surgical biopsy results were reviewed.
Results: 102 pts had homozygous deletion of 9p21 and available follow up (62 males, median age 69yrs). This included 52 pts in UC surveillance and 50 pts with hematuria and no history of UC. Overall, 11 pts developed UC during the follow up period, and all of these pts had a history of UC (21.1%). The 11 UCs included 8 low grade papillary UC and 3 high grade papillary UC. None of these tumors invaded muscularis propria. Four tumors invaded lamina propria. Of these 11 pts, 9 were concurrently FISH+ by amplification. Two pts with isolated 9p21 loss developed UC. One of these pts had a subsequent FISH+ by 9p21 loss and amplification before a non-invasive low grade papillary UC was detected. UC recurrence developed in 19 of 129 (14.7%) FISH negative controls. No hematuria pts developed UC during the study interval.

Table 1: UroVysion Results in Surveillance Patients versus Hematuria Patients

	Bladder Cancer Surveillance	Hematuria
Isolated 9p21 loss	31(2 developed recurrence)	43
9p21 loss and amplification	21(9 developed recurrence)	7

Conclusions: Isolated homozygous 9p21 deletion in UC surveillance pts is associated with a significantly lower risk of recurrence than deletion of 9p21 associated with amplifications of chromosomes 3,7, and/or 17 (9/21 vs 2/31, p=0.0039). Homozygous deletion of 9p21 in pts with hematuria and no history of UC is of doubtful clinical significance, as we detected no UC in these pts. Additional follow up is needed to assess the long term risk of homozygous 9p21 deletion.

307 Review of False Negative UroVysion™ Test on Cytologically Atypical Urine Samples

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Background: Fluorescence in-situ hybridization (FISH) by the UroVysion (UV) system detects cytogenetic changes characteristic of urothelial carcinoma (UC) in urine samples and has been promoted as an ancillary test for urine cytology. In a review of UV testing on cytologically atypical urine, we found a significant number of false negative cases using surgical pathology as a gold standard (USCAP 2007, Poster #272). We seek to determine the cause of the false negative UV testing by manual review of the cases of atypical urine cytology with negative UV testing and low or high-grade UC on surgical biopsy.
Design: There were 25 false negative UV tests out of a total of 176 cases of atypical urine. Slides were available for 18 cases and consisted of Thin Prep and cytospins. The slides were reviewed by two pathologists (RB, KS) and subsequently reviewed jointly. Each case was evaluated for the presence of factors which might lead to a false negative UV test including: only rare atypical cells, degeneration and indeterminate cytology, as well as the presence of inflammation (acute and/or chronic), squamous cells, lubricant, crystals, blood, bacteria, fungus and cellular degenaration. The cases were each scored for the presence or absence of the individual factor.
Results: There was complete agreement between the two pathologists. All 18 cases (100%) were found to have indeterminate cytology, consistent with the prior cytologic diagnosis of atypical urine. Only rare atypical cells were present in 8 (44%) cases. Degenerated cells were present in 5 (28%). Blood was present in 6 (33%) cases. Squamous cells were present in 4 (22%), lubricant in 3 (17%), crystals in 3 (17%), bacteria in 2 (11%) and inflammation in 1 (6%) of cases.
Conclusions: The presence of only rare atypical cells was the strongest association with false negative UV testing in the setting of an atypical urine cytology. This finding suggests that visual inspection may be more sensitive in detecting neoplasia than UV testing. Other factors included the presence of blood, squamous cells, lubricant, crystals, bacteria and acute inflammation, which may have dilutional effects. The findings in this study are observational and limited by the few cases available for review, the findings suggest that caution should be used in interpretting a negative UV result in the setting of an atypical urine cytology. The indeterminate cytology of an atypical urine may warrant surgical biopsy to rule out neoplasia regardless of the UV results.

308 WT1 Is Superior to PAX 2 for Immunohistochemical Detection of Ovarian Serous Papillary Carcinoma in Peritoneal Fluids

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Background: PAX 2 is a homeogene expressed in the urogenital system, including Wolffian and Müllerian ducts and kidneys. Its function as a transcription factor in renal development is well documented, and the PAX 2 immunohistochemical (IHC) antibody has exhibited diagnostic and prognostic utility in epithelial neoplasms of renal origin. The Wilms' tumor (WT1) gene product is consistently detected in normal ovarian germinal epithelium, and ovarian carcinomas frequently exhibit alterations in WT1 function. Serous papillary carcinomas (SPC) of the ovary are strongly immunoreactive for WT1 antibody. Although one recent study showed PAX 2 to be a reasonably sensitive IHC marker for ovarian SPC in tissue samples, data on immunoreactivity (IR) for PAX 2 in tumors of Müllerian origin are sparse, and to our knowlege no previous studies have evaluated its utility in peritoneal fluids. This study was undertaken to compare the IR of PAX 2 to WT1 in metastatic ovarian SPC in peritoneal fluids with the aim of determining the better marker for metastatic SPC.
Design: Conventional sections from 14 cell blocks prepared from peritoneal fluid containing metastatic carcinoma in patients with known SPC of the ovary were immunostained with the following antibodies: 1) PAX 2 (Invitrogen 1/100) utilizing heat pretreatment and Dako polymer envision plus detection system on Dako automated instrument 2) WT1 (Dako 1/50) utilizing heat pretreatment and ultraview detection system on the Benchmark automated instrument. Nuclear IR was considered positive, and extent (% positivity) and intensity (1= light brown, 2 = golden brown through which nuclear chromatin can still be appreciated, 3= dark opaque brown) were evaluated semiquantitatively.
Results: All SPC were uniformly positive for WT1 (100%) with an extent of 80-100% and intensity of 2 to 3. Only 2 of 14 SPC were positive for PAX 2 (14%) with an extent of 2-5% and intensity of 1-2.
Conclusions: Our data suggest that WT1 is a more sensitive marker than PAX 2 for IHC detection of metastatic SPC in peritoneal fluids.

309 HER2/*neu* as a Prognostic Marker in Urine Cytology

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Background: Urothelial carcinoma (UC) is one of the most frequent cancers in the US. Numerous studies have been published on diagnostic markers of UC however prognostic markers have been evaluated much less extensively. The aim of this study is to evaluate HER2/*neu* as a prospective novel biomarkers for UC in urine cytology.
Design: The urinary tract cytologic specimens were prospectively collected from August 2006 to August 2007. Only the cases which had a surgical follow up biopsy or cystectomy were included in the study. A cell block was prepared from the positive cases if sufficient sample was present; and then was evaluated for cellularity and the presence of tumor cells. Immunohistochemical staining for HER2/neu antibody was performed on the cell block sections. The staining pattern of the tumor cells were interpreted according to the previously defined studies on breast cancer. Results of the staining as well as the histomorphologic paramenters, and outcome data was tabulated, and analyzed.
Results: From a total number of 312 urinary specimens 30 were positive for UC of these in 22 cases a cell block was prepared. Four cell blocks were virtually acellular and was excluded from the study. Remainder of the cases stained with HER2/*neu.* The study cases were comprised of 18 cases (16 bladder barbotages, 1 renal pelvis washing and 1 renal pelvis brushing) from pateints ranging from 46-83 years old (mean=58.2) all of whom were males. Ten of the cases were diagnosed on biopsy and a follow up cystectomy. The HER2/*neu* correlations with the various parameters were as follows: age (r=-.33), presence of CIS (r=0.53), depth of invasion (r=-0.04), and lymphovascular invasion (LVI) (r=0.31). There was no correlation between tumor grade and the HER2/*neu* expression.
Conclusions: In our study HER2/*neu* expression was present in most of the UC and was associated with presence of CIS and to lesser extent with LVI. Additional longitudinal studies with help delineate the potential of HER2/*neu* expression in predicting adverse prognostic events.

310 Preservation of Cell Surface Immunophenotype of Lymphoid Lesions during Prolonged Storage of Fresh Fine Needle Aspirations

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Background: Immunophenotyping of cell surface antigens is important in the evaluation of fine needle aspirations (FNA) of lymphoid lesions. Immunophenotyping is performed on fresh samples, however phenotyping may be unavoidably delayed and the effects of storage and delayed phenotyping of fresh samples is largely unknown.
Design: Needle rinses from 10 FNA of lymphoid lesions collected into normal saline underwent ammonium chloride erythrolysis with immediate cell surface immunophenotyping using laser scanning cytometry. Residual fresh sample was then divided and stored at 4°C in either 0.2 mL of phospate buffered saline (PBS) or 0.1 mL PBS supplemented with 0.1 mL of 22% bovine serum albumin. At 24 hours post collection a cell count was performed and an aliquot of $5x10^5$ cells was removed for repeat immunophenotyping and morphological assessment. The residuum was again stored at 4°C with serial immunophenotyping and morphological assessment performed at intervals between 2 to 11 days post collection as the quantity of sample allowed.
Results: The study included 5 cases with clonal B-cell populations, 4 cases with reactive populations and 1 case with an immature T-cell phenotype. With time there was generalized cell loss that was reduced by albumin supplementation. The immunophenotypes obtained during storage were the same as the original phenotypes for both the clonal and reactive populations regardless of albumin supplementation. The phenotypes were still easily discernible after 6 days without albumin and 8 to 9 days in those cases with albumin supplementation and remained unchanged until cell depletion rendered the sample cellularity inadequate for evaluation. With time there was a mild reduction in relative brightness for all surface antigens with CD23 appearing to be the most sensitive. There was a mild relative loss of T-cells and an increase in the CD4/CD8 ratio which was unaffected by albumin supplementation. The presence of the albumin also allowed better resolution of the lymphoid populations in some cases.
Conclusions: Accurate immunophenotypes can be obtained from fresh FNA samples stored at 4°C even after 6 days without the addition of albumin or 9 days with albumin supplementation. Degradation during storage results in generalized cell depletion without alteration of the immunophenotype for both reactive and clonal populations. Addition of albumin to the sample increases sample longevity and may improve population resolution.

311 Atypical Epithelial Cells (AEC) in Bronchoalveolar Lavage (BAL) from Bone Marrow Transplant (BMT) Recipients: A Predictive Feature of Pulmonary Graft-Versus Host Disease (GVHD)?

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Background: In BMT patients, non-infectious pulmonary complication is the major cause of morbidity and mortality. The presence of GVHD is a main contributory factor to this complication. The specific role of BAL cytology in the diagnosis of pulmonary GVHD is not defined. The aim of this retrospective study is to identify specific BAL characteristics of pulmonary involvement in acute GVHD in children.

Design: We reviewed all pediatric patients who presented in our hospital with respiratory complications after allogenic BMT or during chemotherapy alone over a 6 years period (2001 to 2007) and underwent bronchoscopy with BAL. Clinical manifestations of GVHD (in BMT patients only), presence of infection and microbial analyses were recorded. All BAL specimens were reviewed for the presence of AEC using a 4 tier system: 1=negative (without AEC), 2=reactive AEC, 3=atypical cells suspicious for neoplasia, 4=malignant cells. SPSS 11.0 statistical software was used.

Results: 17 patients underwent allogenic BMT, 9 out of 17 presented with respiratory complications and 7 out of them had GVHD. 10 oncology patients treated with chemotherapy alone underwent bronchoscopy for similar complications. 30 BAL specimens (17 from BMT and 13 from chemotherapy patients) were available for analysis. A potential infectious etiology was identified in 7/17 (41%) BMT and 7/13 chemotherapy (54%) BAL specimens (p=NS). 12/17 (70.6%) BMT BAL specimens showed AEC: atypia of grade 2 in two cases and atypia of grade 3 in ten cases. 4/13 (30.8%) chemotherapy BAL specimens showed AEC: atypia of grade 2 in three cases of grade 3 in one case. AEC were significantly more frequent in BMT as compared to chemotherapy BAL specimens (p=0.03), and significantly more severe (p=0.01). The presence and the severity of AEC were not associated with the presence of infection or methotrexate dose and exposure. Moreover, there was a clear trend for a significant association between severe AEC (atypia of grade 3) and severe GVHD (stage III-IV) (p=0.056).

Conclusions: AEC in BMT patients are related to different situations. We showed that AEC are significantly more frequent and more severe in BAL of BMT pediatric patients with pulmonary complications. The trend for an association between severity of atypia and severity of GVHD might support the diagnosis of pulmonary acute GVHD in case of GVHD in others organs.

312 Papanicolaou Test Interpretations of "Atypical Squamous Cells, Cannot Exclude High-Grade Squamous Intraepithelial Lesion": An Investigation of Requisite Duration and Number of Colposcopic Procedures to a Definitive Diagnosis of High Grade Dysplasia in Routine Practice

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Background: The management guidelines for women with papanicolaou (pap) test interpretations of ASC-H (Atypical squamous cells, cannot exclude high-grade squamous intraepithelial lesion) reflect the substantial risk, which ranges from 21 to 68%, of a cervical intraepithelial neoplasia 2 or worse (CIN2+) in their follow-up histologic samples. The present study was initiated to determine the number of colposcopic procedures and the time frame that is typically required for a definitive diagnosis of a CIN2+ lesion following a pap test interpretation of ASC-H in routine practice.

Design: Clinicopathologic data on consecutive ASC-H interpretations, 97% of which were on liquid based preparations, were reviewed. The number of biopies (which was used in this context as a surrogate indicator for the number of colposcopies), as well as the average duration required for a follow-up histologic diagnosis of CIN2+, was determined.

Results: Of 500 ASC-H interpretations, 75 were excluded for a variety of reasons and 165 lacked follow-up in our records. The average patient age and follow-up duration for the remaining 260 patients was 35.6 years and 18.5 months (mo) respectively. CIN2+ was diagnosed in 49 (40%) of the 122 women with at least 1 histologic follow-up. Of these 49 patients, 72% (35/49) were diagnosed on the first follow-up cervical biopsy, 14% (7/49) and 8% (4/49) were diagnosed on the second and third follow-up biopsies respectively; in only 6% was a 4th follow-up biopsy required. Overall, an average of 1.53 biopsies (range 1-4) was required to attain a definitive diagnosis of CIN2+, and 28% of patients required more than 1 follow-up biopsy. The average period between the index ASC-H interpretations and CIN2+ diagnoses was 5.5 mo. The average time to CIN2+ diagnoses by the first follow-up biopsy was 3 mo; for diagnoses made on subsequent biopsies the average *additional* follow up duration was 8 mo. 84% of the eventual CIN2+ diagnoses were rendered within 12 mo of their associated index ASC-H interpretations.

Conclusions: 1) A substantial subset (28%) of patients with biopsy-proven CIN2+ following ASC-H interpretations require more than 1 colposcopy for a definitive diagnosis, and 2) if a CIN2+ lesion is present, the vast majority can be diagnosed in a biopsy performed within 1 year of the ASC-H interpretations.

313 ThinPrep (TP) Cytologic Diagnoses of Low Suspicion Breast Lesions Detected on Screening Ultrasound (US): A Six Year Experience

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Background: Screening breast US is performed by a radiologist in patients who are at high risk for breast cancer but have normal mammographic findings and dense breast tissue. Low suspicion solid-appearing breast lesions undergo biopsy by fine needle aspiration (FNA) and cytologic TP preparation.

Design: We evaluated the clinical outcome in 512 consecutive high risk patients who underwent FNA of breast lesions detected by US, in the absence of mammographic and palpable abnormalities, and correlated the cytology and histology in 138 (27 %) patients who underwent subsequent core biopsies and/or excision. Clinical outcome data was obtained for the remaining 374 (73%) benign cases which were followed by US at six month intervals for two years and annually by mammography. The aspirated material was entirely submitted in Cytolyt for diagnosis on one TP slide and interpreted by one cytopathologist.

Results: Of 512 US-guided FNA biopsies of the breast, 30 (5.8%) were malignant, 28 (5.5%) were suspicious or atypical, 18 (3.5%) were suggestive of papillary lesion, 4 (0.8%) were fibroepithelial lesion-not otherwise specified, and 432 (84.4%) were diagnosed as fibrocystic change or fibroadenoma on TP. All 30 (100%) malignant cases on TP were confirmed by histology to be invasive carcinoma. Histologic follow-up was available in 23 suspicious or atypical lesions on TP. Of these, 15 (65.2%) were invasive ductal carcinoma, 2 (8.7%) were DCIS, 4 (17.4%) were ADH (one within an intraductal papilloma), and 2 (8.7%) were benign without atypia. Of the 18 TP cases suggestive of papillary lesion, histologic follow-up was available in 12, including 7 (58.3%) cases of proliferative fibrocystic change, 1 (8.3%) in situ and invasive ductal carcinoma, 3 (25%) intraductal papillomas and 1 (8.3%) sclerosing papillary lesion. Of 432 cytologically benign cases on TP, there were 84 (19.4%) with surgical biopsy; 82 (97.6%) were benign without atypia, 1 (1.2%) was ADH, and 1 (1.2%) was invasive lobular carcinoma. For statistical calculations, cases in the malignant or suspicious/atypical categories were considered positive for disease. The sensitivity and positive predictive value were both 96%, and the specificity was 98%.

Conclusions: Breast FNA biopsy on TP is highly accurate when the procedure is performed by an experienced radiologist and interpreted by a cytopathologist experienced in breast cytology.

314 E-Cadherin, CD10 and Progesterone Receptor: An Immunohistochemical Trio To Distinguish Pancreatic Endocrine Neoplasm from Solid-Pseudopapillary Neoplasm of the Pancreas

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Background: Cytomorphology for solid pseudopapillary neoplasm of the pancreas (SPN) has been well described. Despite that, SPN continues to pose challenges for cytologists and not infrequently is considered a pancreatic endocrine neoplasm (PEN). Confirmatory immunophenotyping is therefore recommended in difficult cases. On cytology samples where tissue is limited, it is important to select a panel of stains to provide the best confirmatory evidence. Chromogranin immunoreactivity has been consistently noted in PEN but can also be noted in SPN. Therefore, a panel of stains is needed to help distinguish between these two entities. This study was undertaken to identify additional markers to reliably separate SPN from PEN on fine needle aspiration (FNA) samples.

Design: We retrieved 22 endosonographically obtained FNA samples from patients with confirmed PEN (n=16) or SPN (n= 6) with adequate cellularity on cell block preparation. Serial sections were immunohistochemically stained for markers listed in Table 1, chosen after review of recent literature. A chi square test was performed with Yates' correction to determine significant differences in staining patterns between PEN and SPN.

Results: Patients with PEN (8 males: 8 females) had a mean age of 53.9 (range 30-66) years. Of the 16 tumors, 4 (25%) were in the head and the remainder were in the body/tail of the pancreas. Patients with SPN (1 males: 5 females) had a mean age of 33.5 (range 23-43) years. Of these tumors, 16% (1/6) were in the head of the pancreas. Our results show E-cadherin consistently staining PEN but not SPN (87.5% vs.0%; p = 0.001). Progesterone receptor (PR) and CD10 studies were consistently positive in SPN but not in PEN (100% vs. 31%; p= 0.01 and 100% vs. 12%; p=0.001, respectively). Interestingly, in the PEN cases, the positive PR staining was dot-like in appearance in comparison to the SPN cases which stained uniformly.

Conclusions: E-cadherin, CD10 and PR analysis can be used to reliably distinguish PEN from SPN on cytology samples, particularly, in cases where immunohistochemical confirmation is necessary.

Table 1: Positive Immunohistochemical Stain Results

	E-Cadherin	CD56	CD10	PR	Mesothelin	Chromogranin	Synaptophysin
PEN(n=16)	14	7	2	5	4	15	13
SPN (n=6)	0	6	6	6	3	3	4
p value	0.001	0.057	0.001	0.0167	0.4168	0.08	0.4

315 Cytological Evaluation of Image-Guided Fine Needle Aspiration Biopsies Via Robotic Microscopy: A Pilot Validation Study

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Background: Image-guided fine-needle aspiration biopsy (FNAB) has been increasingly recognized as the choice of diagnostic tool in the management of deep-sited masses. On-site immediate assessment is one key component in the FNAB, which assures adequate sampling, guides specimen triage for appropriate work-ups and in some cases renders a diagnosis for immediate therapeutic intervention. Although tele-pathology is being integrated into surgical pathology practice, including intraoperative diagnosis, consultation service, and quality control, its role in FNAB has not been explored. In this pilot study, we examine the feasibility of cytological evaluation of image-guided FNAB via robotic microscopy.

Design: Total forty cases of image-guided FNAB of lung, liver, pleural, or mesentery masses with benign (n = 5) or malignant (n = 35) diagnosis were retrieved from the cytopathology archives at the University of Pittsburgh Medical Center-Shadyside Hospital. Representative Diff-Quick and Papanicolaou-stained slides were reviewed via robotic microscopy followed by real slide assessment. The cytological evaluations that included sampling adequacy assessment and cytological diagnosis were compared between the two approaches and among the individual cytopathologists. The intra- and inter-observer discrepancies were analyzed and resolved by follow-up consensus conference.

Results: For sampling adequacy assessment, there were high correlation rates (intraobserver: 95%, interobserver: 92.5%) between the two approaches, with few discrepancies being between suboptimal vs. satisfactory. Analysis of diagnostic interpretation showed correct classification of 93% (intraobserver) or 88% (interobserver) of benign and malignant cells, with the discrepancies being between benign and atypical cells in the benign group, and suspicious and malignant diagnosis in the malignant cases. Within the malignant group, 77% of cases were accurately subclassified via the robotic imaging approach. Overall image quality was considered satisfactory in 95% of the slides.
Conclusions: The results demonstrated that cytological evaluation of FNAB slides via robotic microscope was feasible, with few problems raised. The main problems included longer evaluation time, particularly in those cases with thick bloody smears and low tumor cellularity, and difficulties in recognizing neuroendocrine differentiation and hepatocellular carcinoma.

316 Improved Accuracy for Detection of Urothelial Carcinoma Using FISH Compared to Cytology: A Clinicopathological Study of 1,006 Cases

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Background: Urothelial carcinoma (UC) is associated with a significant risk of recurrence and progression of invasive disease, and therefore, patients with UC need surveillance testing. This study evaluates the use of fluorescence in-situ hybridization (FISH) for detecting new and recurrent UC in a cancer center.
Design: From 2004 to 2006, 1006 consecutive urinary specimens from 600 patients with a mean age of 66 years (M:F 448:152) were monitored for recurrent UC (712 cases) or evaluated for urinary symptoms (294 cases). Patients were evaluated by cytology and the UroVysion FISH containing probes for chromosomes 3, 7, 9p21, and 17. In each case, 25 cells were analyzed by 2 observers and a positive FISH test contained 4 or more abnormal cells for 3, 7, and 17 or 10 or more abnormal cells with 9p21 deletion. The FISH results were correlated with the cytologic findings and clinical follow-up (F/U) including cystoscopy (359 cases), biopsy (423 cases), and F/U cytology (101 cases) ranging from 6 to 24 months (mean, 12.3). For statistical purposes, clinical follow-up was considered positive if cystoscopy or biopsy was positive.
Results: On FISH analysis, there were 672 negative, 271 positive, and 63 unsatisfactory cases. The cytology diagnoses were negative (541 cases), atypical (215 cases), positive for malignancy (152 cases), and suspicious (98 cases). The overall sensitivity and specificity for FISH were 62% and 86%, respectively. For cytology, the sensitivity and specificity were 54% and 86%, respectively.

Correlation of FISH results with cytology and clinical F/U

	Cytology			HX of UC	Clinical F/U		
	Neg/Aty	Susp	Pos	No / Yes	Neg	Pos	N F/U
FISH (neg) - 672	643	27	2	199 / 472	533	103	36
FISH (pos) - 271	53	69	149	72 / 199	91	170	10

*Insufficient cases excluded; Neg, negative; Pos, positive; Aty, Atypical; Susp, suspicious; NF/U, no follow-up

Conclusions: FISH on urinary specimens has been shown to be sensitive and specific for UC. The sensitivity for FISH is higher than cytology and FISH can detect recurrence (anticipatory positives) earlier than other methods such cytology and cystoscopy.

317 Broom Versus Broom-and-Brush: A Comparison of SurePath® Liquid-Based Papanicolaou Test (LBPT) Collection Devices

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Background: The use of endocervical cells (EC) to determine Pap test adequacy is controversial. Many clinicians currently rely on ECs as a marker of adequacy, and concerns exist that use of the SurePath® broom as a sole collection device might decrease diagnostic yield. In response to these concerns, the maker of the SurePath® LBPT system obtained additional FDA clearance for the use of a spatula in combination with an endocervical brush. A small study (Day et al, 2004) did not find any improved diagnostic utility when additional collection devices were used in conjunction with the SurePath® broom. The aim of this study is to determine if these findings are reproducible in a larger population.
Design: We prospectively collected data on sampling devices received within the LBPT specimen container at the point of accessioning in our multi-institutional laboratory between 4/27/07 and 7/2/07. We compared the performance of the SurePath® broom and the endocervical brush, used alone or in combination.
Results: Of 9858 consecutive LBPT samples accessioned, 1181 were excluded due to lack of device data or failure of the final report to comment on the presence of EC component. 16 of the remaining 8677 cases were unsatisfactory. The study population had a mean age of 40.3 ± 14.3 years. Of the 8661 satisfactory LBPTs, 635 (7.3%) had an abnormal diagnosis. The combination of Broom + Brush (B+B) increased EC recovery compared to Broom alone ($p<0.001$). This did not correspond to a significant increase in diagnostic yield.

Collection device	n	Average age (+/- SD)	Unsatis-factory	EC present	ASCUS + ASC-H	LSIL + LSIL-H	HSIL
Broom	7465	39.8 +/- 14.1	10 (0.1%)	5135 (68.9%)	354 (4.7%)	156 (2.1%)	22 (0.3%)
Brush	97	41.7 +/- 16.3	2 (2.1%)	62 (65.3%)	2 (2.1%)	2 (2.1%)	1 (1.1%)
Broom + Brush	1092	43.4 +/- 14.4	1 (0.1%)	837 (76.7%)	55 (5.0%)	20 (1.8%)	4 (0.4%)
None present	23	43.7 +/- 18.9	3 (13.0%)	9 (45.0%)	3 (15.0%)	0 (0.0%)	0 (0.0%)
Total	8677	40.3 +/- 14.3	16 (0.2%)	6043 (69.8%)	414 (4.8%)	178 (2.1%)	27 (0.3%)

Conclusions: The use of B+B increases EC yield but does not increase the rate of abnormal diagnoses. However, the slight increase in HSIL diagnoses observed in the B+B category, while not significant, may justify larger studies.

318 Can ThinPrep (TP) Preparations Be Used in the Primary Mode in the Diagnosis of Pancreatic Ductal Adenocarcinomas in Material Obtained from Endoscopic Ultrasound Guided Fine Needle Aspirations (EUSFNA)?

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Background: In fine needle aspiration cytology (FNAC), TP is frequently used in the adjunct mode. The aims of this study were to see whether 1) if the previously published smear based cytological criteria are applicable to TP preparations when used as the primary mode of specimen preparation in cases of pancreatic ductal adenocarcinoma (PDAC) undergoing EUSFNA and 2) utilization review (UR) of lab resources by direct smears (DS) vs. TP.
Design: Forty-eight histologically confirmed PDACs for which EUSFNA had been performed and TP slides were available for review were retrieved. The slides were evaluated semi-quantitatively for 11 parameters, retrospectively. UR of the time used for adequacy check and number of slides generated by the smear technique was also performed in 34 cases prospectively.
Results: The semi -quantitative analysis results of cytological criteria are summarized in Tables 1 and 2.

Table1. Prevalence of Criteria (1-3) in ThinPrep of EUSFNA

Score	0 (%)	1(%)	2(%)	3(%)
Cell size variation (Score1-2)	-	29/48 (60)	19/48 (40)	-
Nuclear membrane irregularity (Score 0-2)	3/48 (6)	41/48 (85)	4/48 (9)	-
Cellularity (Score1-3)	-	22/48 (45)	18/48 (37)	8/48 (18)

Scores for cell size variation: 1: ≥ 2 times; 2: ≥ 4 times. Scores for nuclear membrane irregularity: 0: no irregularity; 1, nuclear notch or groove; 3, popcorn cells or raisinoid. Scores for cellularity: 1, ≤ 2 clusters/10X; 2, 3-5 clusters/10X; 3, ≥6 clusters/10X

Table 2.Prevalence of the Cytologic Criteria (4-11) in ThinPrep of EUSFNA

CF	**Prevalence (%)**	**CF**	**Prevalence (%)**
Three-dimensionality	26/48 (54)	Macronuleoli	11/48 (23)
Necrosis	21/48 (44)	Mucin vacuoles	11/48 (23)
Irregularly placed nucleus	22/48 (46)	Multinucleation	6/48 (13)
Chromatin clearing	19/48 (40)	Mitosis	0/48 (0)

CF: Cytological Feature

The results of UR data in EUSFNA DS are as follows: 1) Average time for adequacy check, 88 (30- 150) min. for DS vs. 0 for TP; 2) Average # of slides, 11 (3-32) for DS vs. 1(1-2) + Cell Block for TP; 3) Unsatisfactory rate, 15% for DS vs. 1.8% for TP.
Conclusions: The data from this study suggests that TP can be an efficient primary preparation mode in EUSFNA of the PDACs. UR data clearly demonstrates the advantage of TP over DS without compromising adequacy. Known smear based cytological criteria are well represented in TP.

319 HPV DNA Detection in ThinPrep Pap Test Vials Is Independent of Cytologic Sampling of the Transformation Zone in HSIL Pap Tests and Subsequent Biopsy Diagnosis

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Background: Sampling of TZ/EC has been regarded as a quality indicator in screening cervical abnormalities; however, the significance of a TZ/EC in promoting disease detection remains controversy. Data on the correlation of TZ/EC absence, hrHPV DNA detection, and histologic follow-up are very limited.
Design: The computerized records of MWH were searched for cases that were diagnosed as HSIL in ThinPrep Pap tests (TPPT) and that also had hrHPV DNA testing by HC2 from 7/1/2005 to 7/31/2007. The presence or absence of a TZ/EC and histologic follow up results were obtained. Average follow-up period was two months (0-18 months). Fisher's exact test was performed by SAS 9.1.
Results: 133 women had TPPT with HSIL interpretations and also had hrHPV DNA testing during the study period. hrHPV DNA prevalence showed no significant difference between women with and without TZ/EC (table 1). 100 women (75.2%) had histologic follow up. The percentage of SIL on histologic follow-up was not statistically significant between women with and without TZ/EC in their preceding TPPT (table 2).

Table 1. hrHPV prevalence in women with HSIL Paps

		TZ present			TZ absent		
Age	HPV tested	Positive (%)	95% CI	HPV tested	Positive (%)	95% CI	P
10-	9	9 (100)	100-100	0			
20-	42	41(97.6)	93.0-100	4	4(100)	100-100	0.9130
30-	46	41(89.1)	80.1-98.1	2	2(100)	100-100	0.8005
40-	14	13(92.9)	79.5-100	0			
50-	8	6(75.0)	45.0-100	1	1(100)	100-100	0.7778
60-	4	3(75.0)	32.6-100	3	2(66.7)	13.4-100	0.8571
All	123	113(91.9)	87.1-96.7	10	9(90.0)	71.4-100	0.8060

Table 2. Histologic SIL in women with positive hrHPV

		TZ present			TZ absent		P
Age	CIN2-3 (%)	CIN1 (%)	SIL (%)	CIN2-3 (%)	CIN1 (%)	SIL (%)	
<30	20 (54.1)	14 (37.8)	34 (91.9)	1(50.0)	1(50)	2(100.0)	0.8502
>29	33 (68.8)	12 (25.0)	45 (93.8)	3 (75.0)	1(25.0)	4(100.0)	0.7826
Total	53 (62.4)	26 (30.6)	79 (92.9)	4 (66.7)	2(33.3)	6(100.0)	0.6561

Conclusions: HSIL TPPT with and without TZ/EC had a comparable hrHPV DNA detection rate and very similar subsequent surgical follow up results. hrHPV DNA detection in TPPT vials is independent of cytologic sampling of the TZ/EC.

320 HPV Prevalence and Type Distribution in Young Women with Negative Cytology

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Background: High-risk (HR) HPV types 16 and 18 account for more than 70% of invasive cervical carcinomas (ICC) worldwide and are the most common types detected in high-grade cervical intraepithelial neoplasia (CIN). Accordingly, vaccination of girls

and young women against HPV16/18 is expected to result in a lowered incidence of ICC and CIN2/3. Vaccination against HPV6 or 11 may prevent genital warts. This study has investigated the distribution of HPV types in cytology samples diagnosed as negative for an epithelial lesion or malignancy (NILM) to examine the early exposure of young women in Vermont to HPV16, 18 and other HPV types.

Design: DNA was extracted, purified and quantified from 141 NILM samples collected from young women (age range: 14-24 years, mean age: 19.5, SD: 2.42) participating in cervical screening. HPV testing was performed by GP5+/6+ PCR (two alternative assays) and by PGMY09/11 PCR. HPV type was characterized by dot-blot hybridization or cycle sequencing.

Results: Twenty-three different HPV types were detected in 49/141 (34.8%) samples. HR HPV was identified in 32/141 (22.7%) women, and HPV16 or 18 in 26/141 (18.4%) specimens. Low-risk (LR) HPV types were detected in 17/141 (12.1%) samples. HPV types 6 or 11 were uncommon; just one infection was detected. Multiple infections were identified in 8/141 (5.7%) specimens. Significant differences were found comparing teenagers with women aged 20 to 24 years (Table 1).

Table 1

Age Range	14-19 years	20-24 years	
N	75	66	P Value
Mean Age (SD)	17.59 (1.34)	21.74 (1.10)	<0.0001
HPV	18 (24.0%)	31 (47.0%)	<0.005
HR HPV	11 (14.7%)	21 (31.8%)	<0.02
HPV16/18	9 (12.0%)	17 (25.8%)	<0.05

Conclusions: Sub-clinical HPV infections are widespread amongst young women: 47% aged 20-24 and 24% aged 14-19 tested positive; HPV16 and 18 were the most common infections. These data demonstrate that HR HPV exposure occurs at an early age supporting current vaccination policy. The development of additional vaccines is suggested by the finding that 6/32 (18.8%) HR infections were positive for types other than 16 or 18. These data may be useful for improving public health awareness of HPV infections, both for promoting protective behavioral measures and HPV vaccination availability. Patient follow-up is required to determine the constancy of the infections detected, subsequent cytological diagnoses, and the potential clinical utility of HPV screening NILM samples.

321 The Value of Cytogenetics and Molecular Cytogenetics as Adjuncts to Cytology in the Diagnosis of Malignant Mesothelioma in Pleural and Peritoneal Fluids

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Background: Malignant mesotheliomas exhibit clonal chromosomal aberrations that vary from one tumor to another, but common patterns, such as deletions in 1p, 3p, 6q, 9p, and 22q, have been observed. Because these deletions are absent in normal or reactive mesothelial cells, they can be used to confirm a diagnosis of mesothelioma. This retrospective study investigates the utility of two methods, karyotyping and fluorescence in situ hybridization (FISH), as adjuncts to conventional cytologic examination in patients suspected of having mesothelioma.

Design: We performed a retrospective analysis of 59 pleural or peritoneal fluids from patients suspected of having mesothelioma. An attempt was made to obtain a karyotype in all cases. In 36 cases (61%), FISH for 9p (*p16* gene) and 22q deletions was performed because the karyotype was normal or unsuccessful.

Results: 51 specimens (86%) had a biopsy-proven diagnosis of malignant mesothelioma and 8 had a negative biopsy. Of the 51 fluid specimens from patients with biopsy-proven mesothelioma, a karyotype was obtained in 39 cases (76%). 19 karyotypes (49%) were abnormal and 20 (51%) were normal. In the mesothelioma cases where FISH was performed, 23 (67%) demonstrated an abnormality and 13 (32%) did not. A karyotype and FISH were both performed in 27 cases (53%). 8 (30%) were abnormal by both studies; 10 (37%) had a normal karyotype but an abnormal FISH result; and 8 (30%) cases were negative by both. Of the 8 specimens from patients with negative biopsies, 7 had a negative karyotype and/or FISH result and 1 had an abnormal karyotype. Of the cases with biopsy-confirmed mesothelioma, 25 (49%) had a cytology result reported within five months of the biopsy. The cytology diagnosis was negative or suspicious in 13 (52%) cases; of these, 8 (32%) had an abnormal karyotype or FISH result.

Conclusions: Cytogenetic and molecular cytogenetic analysis of effusions is a useful adjunct to cytology in the diagnosis of mesothelioma by confirming the presence of genetic aberrations in cases that are negative or suspicious by cytology.

322 EUS-GUIDED Fine Needle Aspiration Biopsies Processed with Thin Layer Cytology for Pancreatico-Biliary Solid Lesions: A Single Center Experience

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Background: Endoscopic ultrasound-guided fine needle aspiration (EUS-FNA) has improved the efficacy of the diagnosis of bilio-pancreatic solid lesions. Although in the majority of centers EUS-FNA conventional smears (CS) are the preferred choice for processing cytologic samples thin-layer cytology (TLC) is becoming a reliable option for non-gyn specimens, even if a limited experience is available. TLC may achieve a diagnostic sensitivity as high as CS and immunocytochemistry (ICC) can be reliably performed on TLC as accurately as CS.

Design: During the year 2006 86 patients with solid lesions of the pancreas, bile ducts and hepatic hilum underwent EUS-FNA, performed using a linear array echoendoscope (GF-UC140P, Olympus USA) with a 22G disposable needle. The aspirated material was placed into a preservative solution (Cytolyt, Cytyc, Boxborough, MA) and processed using ThinPrep 2000. 31 cases underwent surgery; in the other cases clinical follow up established the definitive diagnosis.

Results: TLC on EUS FNA yielded malignant in 57 cases (66.3%), benign in 7 (8%), atypical in 4 (4.6%) and inadequate in 18 cases (20%). In 10 cases (11.6%) the diagnosis was supported by ICC carried out on TLC with 7 neuroendocrine neoplasms and 3 non-Hodgkin lymphomas correctly diagnosed. Twenty-one malignancies referred to surgery were histologically confirmed. Out of 4 benign diagnoses, 3 were malignant and 1 was histologically confirmed. Six inadequate resulted as 5 malignancies and 1 benign lesion. The overall diagnostic accuracy was 88%, sensitivity 87.5%, specificity 100%, PPV 95.5% and NPV 100%.

Conclusions: TLC is a valid method for processing EUS-FNAB from bilio-pancreatic solid lesions. Additional investigations (ICC) are easily applicable on TLC. Future comparative studies with CS will be necessary to establish the diagnostic role of this technique. Fadda G. et al. Acta Cytol 2006; 50:129. Siddiqui MT et al. Cancer Cytopathol 2003; 99:205.

323 Reflex Urovysion® Testing in Suspicious Urine Cytology Cases. A Major Cancer Center Experience

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Background: Urovysion® is a FDA approved FISH probe set for use in monitoring urothelial carcinoma (UC). It has a reported sensitivity ranging from 65 to 73% for pTa lesions and 95-100% for pT1-T4 UC which is higher than cytology. However, Urovysion® is an expensive and time-consuming test. The objective of our study is to evaluate the usefulness of Urovysion® as a reflex test in patients with suspicious cytology diagnosis, in an attempt to maximize the costs and staff time. The rationale is that a patient with suspicious cytology and positive Urovysion® test should be submitted to further work up including cystoscopy and radiologic studies.

Design: The study population included 87 urine specimens diagnosed as suspicious over a period of 6 months. As a reflex procedure, the remainder of the material sent to cytology was submitted for Urovysion® test. The Urovysion was reported as positive or negative according to the manufacturer's criteria, although the presence of any abnormality was recorded. The cases with histological follow-up were used to calculate sensitivity, specificity, negative and positive predictive value.

Results: Forty-five of 87 cases had histological follow-up. They were represented by 31 voided and 14 instrumented urine specimens. The results using the reporting criteria suggested by the manufacturer are listed in table 1. The sensitivity was 59%, the specificity was 50%, the positive predictive value (PPV) was 88% and the negative predictive value (NPV) was 16%.

Table 1: Results using manufacturer's criteria

Urovysion result	Positive Biopsy	Negative Biopsy
Positive	23	3
Negative	16	3

The results using the presence of any abnormality as a positive test showed a sensitivity of 80%, specificity of 33.3%, PPV of 89% and NPV of 22%. The results using this criteria are listed in table 2.

Table 2: Results considering any cytogenetic abnormality as a positive test

Urovysion result	Positive Biopsy	Negative Biopsy
Positive	32	4
Negative	7	2

Conclusions: The presence of cytogenetic abnormalities in the Urovysion test in a case diagnosed cytologically as suspicious is highly predictive of urothelial carcinoma. However, a negative Urovysion test does not rule out the presence of urothelial carcinoma. The use of less strict criteria dramatically increases the sensitivity of Urovysion without affecting PPV.

324 Utility of High Risk HPV DNA (hrHPV DNA) Testing in Women with Atypical Glandular Cells

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Background: The cytologic interpretation of atypical glandular cells (AGC) has limited reproducibility. AGC paradoxically is often reported in association with benign conditions, even while AGC is also associated with a significant risk of neoplasia. Whereas reflex hrHPV DNA testing following ASC-US Pap results has been established as improving the efficiency of follow-up for patients with atypical squamous abnormalities, guidelines on the use of hrHPV DNA testing in patients with AGC Pap results are still evolving. This study was carried to further evaluate the usefulness of hrHPV DNA testing in women with AGC Pap results.

Design: The computerized records of Magee-Womens Hospital (MWH) were searched for cases interpreted as AGC from 6/1/2005 to 3/31/2007. Cases of AGC with hrHPV DNA testing by HC2 on residual vial fluid and subsequent surgical follow-up were included in this study.

Results: A total of 519 women with AGC had surgical pathology follow-up. hrHPV DNA test results from residual vial fluid were available in 216 (42%) cases. The overall prevalence of hrHPV was 23% in women with AGC Paps. The correlation of hrHPV DNA testing result and histologic findings are shown in Table 1.

Table 1. hrHPV DNA testing result and histologic diagnosis in wmen with AGC Pap

Histologic diagnosis	HPV positive n=50	HPV negative n=166	Total n=216
Squamous neoplasia	28 (56.0%)	29 (17.5%)	57 (26.4%)
CIN2/3	8* (16.0%)	1 (0.6%)	9 (4.2%)
CIN1	20 (40.0%)	28 (16.8%)	48 (22.2%)
Cervical glandular neoplasia	7 (14.0%)	0	7 (3.2%)
AIS	7* (14.0%)	0	7 (3.2%)
Endometrial lesions	0	12 (7.2%)	12 (5.6%)
Endometrioid Ca	0	5 (3.0%)	5 (2.3%)
Endometrial hyperplasia	0	7 (4.2%)	7 (3.2%)

* 3 patients had co-exisiting AIS and CIN2/3

Conclusions: 1. hrHPV DNA detection was strongly associated with histologic precancerous cervical squamous and glandular follow-up findings (CIN2/3 and AIS). 2. Negative hrHPV DNA results were present in all women with AGC Paps who on

histologic follow-up had endometrial hyperplasia or endometrial cancer. 3. Age and population demographics significantly impacted likely histologic follow-up after. Malignant and precancerous endometrial lesions predominated in women 50 and older, whereas CIN2/3 lesions predominated in women less than 40. All AIS patients were younger than 50. 4. These data suggest that positive reflex hrHPV DNA test results in women with AGC Pap findings are most useful in assisting in detection of precancerous squamous and glandular lesions (CIN2/3 and AIS) in women younger than 50.

325 Clinical Significance of Atypical Glandular Cells in Pap Smears: A Histologic Follow-Up Study from an Older Age Demographic Population

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Background: The Pittsburgh metropolitan area has one of the older age demographic profiles of comparable size U.S. cities. The aim of this study was to analyze atypical glandular cell (AGC) cases and the incidence of clinically significant lesions on histologic follow-up in a large academic practice with a high prevalence of older women.

Design: The computerized records of our hospital were searched for cases diagnosed as AGC from 6/1/2005 to 3/31/2007. The cases of AGC were classified into six groups (table 1). The histologic diagnoses were correlated with the cytologic diagnoses.

Results: Among 198,027 Pap smears including 191,868 (96.9%) ThinPrep Pap tests and 6159 (3.1%) conventional smears examined during the study period, AGC was reported in 818 cases (0.41%), with tissue follow-up available in 519 cases (63.5%). The average follow-up period was 2.6 months (6 days to 24 months). The cytologic and histologic correlations of these 519 cases are shown in Table 1.

Table 1

Cytologic dx	AGC/ ASC-US	AGC/ ASC-H	AGC/ HSIL	AGC-EM	AGC-EC	AGC-NOS
Histologic dx	94	29	26	82	104	184
Invasive squamous Ca			1			
CIN2,3	2	2	15		9	7
CIN1	15	9	8	1	35	28
AIS		2	5		4	2
Endometrial Ca	6		1	25	1	15
ACH				6	1	1
Complex hyperplasia	2			4		1
Ovarian serous Ca				1	1	1
Endometrial polyp	11			13	8	14
Endocervical polyp	1		1	2		5
Endocervical tubal metaplasia	7			5	7	25
MGH	2	1		5	4	7
Average age	46	39	39	54	39	49

7 women had co-existing AIS and CIN2,3

Conclusions: 1.Significant pathologic lesions, defined as carcinoma, AIS, CIN, and endometrial hyperplasias, were identified in 189 of 519 women (36.4%). 2. Neoplastic glandular lesions, defined as invasive adenocarcinoma, AIS, and complex atypical endometrial hyperplasia, were identified in 53 of 519 women (10.2%). 3. Cervical precancer, defined as CIN2,3, and AIS was detected in 41 of 519 women (7.9%). The lower prevalence of CIN2,3 in this series compared to other reported series reflects the older demographics of the patient population. 4. A diagnosis of AGC-EM was the most clinically significant subtype of AGC, with 24.4% cases showing neoplastic endometrial lesions. 5. Among benign lesions, endometrial polyps and endocervical tubal metaplasia were most common (17.4%). 6. Patient age and population demographics significantly impact AGC histologic follow-up. Malignant and precancerous endometrial lesions predominate in women 50 and older, whereas cin2,3 lesions predominate in women less than 40 and all AIS patients are younger than 50.

326 Use of a Limited Immunocytochemical Panel in Classification of Malignant Pleural Effusions in Women

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Background: The majority of the patients with malignant effusions have a known history of carcinoma; however in approximately 8-10% cases, the source of origin is unknown. Carcinomas of the breast and lung are commonly seen neoplasms in women who present with malignant pleural effusion. The aim of this study is to evaluate the use of a limited immunocytochemical panel in subclassification of malignant effusions in female patients.

Design: Of 950 pleural fluid specimens collected over a 3-year-period, 140 were diagnosed as malignant effusions, 81 affecting women. Metastatic carcinomas were proven by histology or clinical follow-up in 71 women. Of those, fifty-two cases (70%) were of breast or lung origin. The original Papanicolaou-stained, cytocentrifuged slides were available in forty cases (28 breast, 12 lung). The carcinoma cells were diamond dotted. The slides were stained with monoclonal TTF-1 (8G7G3/1, DAKO) and ER (ID5, DAKO) antibodies using LSAB detection system (DAKO, Carpinteria, CA) without destaining. Retrospective review of the correspondent histology and immunohistochemistry results (17 breast, 2 lung) was performed.

Results: TTF-1 was expressed in 11 of 12 effusions of from patients with lung adenocarcinomas (92%). ER was positive in 21 metastatic effusions from 28 known breast carcinomas (75%). Both antibodies showed intense nuclear staining in isolated and groups of tumor cells. There was 100% correlation between the immunocytochemical staining of ER and TTF-1 antibodies and the immunoreactivity for these antibodies in the corresponding tissue sections. There was no cross-reactivity between these two antibodies in any of the samples.

Conclusions: A limited immunocytochemical panel of TTF-1 and ER, is helpful for subclassification of the two common carcinomas in women i.e. breast and lung involving pleural cavity. Furthermore, using 8G7G3/1 clone for TTF-1 and ID5 antibody for ER in pleural effusions, there was a 100% concordance with the primary carcinomas in histologic sections. In cases of metastatic carcinomas from breast and lung origin, these two markers confirm the site of origin. In addition, ER may also serve as a marker for specific therapy in patients with remote history of breast cancer, when only archival cytologic material is available.

327 Diagnosis and Typing of Systemic Amyloidosis. The Role of Abdominal Fat Pad Fine Needle Aspiration Biopsy (FPFNA)

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Background: Systemic amyloidosis (SA) is characterized by multiorgan involvement resulting in broad nonspecific presentations. FPFNA has been reported to be excellent for diagnosing SA, with 85-100% specificity, 55-88% sensitivity, and 100% positive predictive value. These results may not be universal and may be limited in terms of SA typing.

Design: Thirty-seven FPFNA from 36 patients in a large academic hospital during a 15-year period were reviewed. There were 16 women and 20 men (age range of 44- 88 years). Smears were stained with Diff-Quik, Papanicolaou and Congo red (CR) stains. Cell-blocks were stained with CR. Phenotyping using antibodies against serum amyloid protein, serum amyloid A, albumin, pre-albumin, kappa light chain, and lambda light chain was done on 6 cell blocks from 5 patients with confirmed diagnoses. Clinical and histological follow-up for each patient was correlated with the FPFNA findings.

Results: FPFANs were positive for amyloidosis, confirmed by CR stain, in 4/36 (11%), suspicious in 1/36 (3%), negative in 26/36 (72%), and inconclusive due to insufficient material in 5/36 (14%). In each of the positive 4 patients, SA was confirmed within 2-16 weeks by tissue biopsies or serum protein electrophoresis. The suspicious case had multiple myeloma, but SA was not confirmed by additional studies. Among 26 patients with a negative FPFNA, SA was finally diagnosed in 21 by tissue biopsies, with the other 5 patients being lost to follow-up. Among the 5 patients with insufficient FPFNA, SA was diagnosed in 4 by biopsies or serum electrophoresis, and one was lost to follow-up. Specificity was 100% whereas sensitivity was 13%. SA typing was successful in three, uninterpretable in one, and negative for all the antibodies in two cases.

Conclusions: FPFNA for diagnosing SA is not as good as previously reported. This discrepancy may be explained by different medical practice setting, e.g., general hospital vs specialized center, low prevalence of SA in general population (1/60,000), low number of procedures (36 in 15 years in this study), or absence of abdominal fat tissue involvement in SA. A negative result of FPFNA does not exclude the diagnosis of SA. Immune phenotyping of amyloid is possible on cell block material.

328 Transbronchial Fine Needle Aspiration (TFNA) Preparation Methodology: How Does Liquid Based (ThinPrep) Processing as the Primary Mode without Adequacy Evaluation Compare with Direct Smears?

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Background: TFNA has evolved as an important tool for diagnosing lung masses and for staging patients with lung cancer. The method of preparation is crucial to maximize the diagnostic yield. This study compares the two methods: Direct smears (DS) vs ThinPrep (TP).

Design: TFNA performed from January 1999-August 2007 were identified from the archives of a large academic hospital and reviewed. Subsequent surgical specimen reports, including resection or biopsy, were also reviewed.

Results: Ninety-six TFNA from 88 patients were identified. Patients age ranged from 17-87 years (mean 64). Forty-nine patients were men and 39 women. Thirty-four specimens were prepared by TP technique whereas 62 were DS. The average number of slides by DS was 9 compared to only 1 by TP. Cell block was utilized for additional diagnostic studies such as immunohistochemistry in 19 TP cases. Some DS required additional time for on-site adequacy checks but these were not required for TP. Using TP technique sensitivity was 43% and specificity 100% in comparison to 68% sensitivity and 94% specificity for DS. The unsatisfactory rate was 14.7% for TP and 6.5% for DS. The unsatisfactory rate for TP by an experienced pulmonologist was 9% compared to 25% for others.

Conclusions: Our study demonstrates that 1) TP and DS methods have comparable specificity but DS appears to have higher sensitivity albeit the numbers are small for TP. 2) Fewer number of slides were prepared for TP without the need to perform adequacy checks on site. 3) Ability to consistently make a cell block and do special studies comparable to a tissue biopsy was available for TP but not DS. 4) TP had a higher unsatisfactory rate. However, this appears to be operator dependent.

Results of TP method

Cytology Diagnosis	Positive Histology	Negative Histology	No Histology	Total
Positive	3	0	5	8
Negative	4	6	8	18
Suspicious	0	0	3	3
Unsatisfactory	1	1	3	5

Results of DS method

Cytology Diagnosis	Positive Histology	Negative Histology	No Histology	Total
Positive	14	1	2	17
Negative	9	16	11	36
Suspicious	5	0	0	5
Unsatisfactory	0	1	3	4

329 Rabbit Monoclonal Antibodies in Detection of ER, PR and Her2 in Breast Carcinoma Cell Block Sections by Conventional Microscopy and Quantitative Image Analysis

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Background: Accurate assessment of estrogen receptor (ER), progesterone receptor (PR) and Her2 status of breast carcinomas is critical, given the implications for predicting response to systemic therapies.Recently developed rabbit monoclonal antibodies (RMab) are reported to have higher sensitivity than murine monoclonal antibodies (Mab). This study compares RMabs against FDA-approved Mab (FMab) in breast carcinoma cell block sections using visual (V) and image (I) quantification of marker expression.

Design: Cell blocks of 52 breast cancers were studied. Immunohistochemistry was performed, using RMab (Lab Vision): ER (SP1; 1:100), PR (SP2; 1:400), Her2 (SP3; 1:100). FMab (Dako) was used as the gold standard: ER (1D5; 1:50), PR (PgR636; 1:400), Herceptin kit and fluorescent in situ hybridization (FISH) for Her2. Visual scoring by light microscopy was done using <5% as negative for ER and PR, and 0 and 1+ as negative for Her2. These slides were analyzed with ACIS III (Dako). McNemar's statistical analysis was done and kappa coefficients were calculated.

Conclusions: Although there is fair-poor agreement between RMab versus FMab for ER and PR expression, the difference is not statistically significant. Comparing visual versus image methods, significantly higher positivity rates were noted with image analysis. For Her2 overexpression, FMab proved to be superior to RMab and showed excellent agreement with FISH results especially with visual analysis.

Results using RMab and FMab, visual and image methods

Detection Method	Pos. agreement %	Neg. agreement %	Kappa	P value
ER VRMab/ VFMab	60	78.1	0.3	0.8
ER IRMab/ IFMab	79.3	69.6	0.5	0.8
ER VRMab/ IMRab	46.7	77.3	0.2	0.02
ER VFMab/IFMab	65.5	95.7	0.6	0.007
PR VRMab/VFMab	86.7	83.8	0.7	0.1
PR IRMab/ IFMab	61.5	57.7	0.2	0.8
PR VRMab/IRMab	59.2	88	0.5	0.03
PR VFMab/IFMab	53.9	96.2	0.5	0.002
Her2 VRMab/VFMab	28.6	100	0.2	0.0001
Her2 IRMab/IFMab	12.5	69.2	0.07	0.09
Her2 VRMab/ IRMab	75	91.2	0.2	0.002
Her2 VFMab/IFMab	62.5	61.5	0.2	0.06
Her2 VRMab/FISH	80	100	0.9	0.1
Her2 VFMab/FISH	100	4.6	0.08	0.0001
Her2 IRMab/FISH	30	72.7	0.3	0.005
Her2 IFMab/FISH	42.9	61.5	0.2	0.2

330 The Utility of MART-1 / Melan-A in Distinguishing between Metastatic Melanoma and Renal Cell Carcinoma

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Background: MART-1, also called A103 / Melan-A is a **M**elanoma **A**ntigen **R**ecognized by autologous cytotoxic **T** lymphocytes from melanoma patients. It is known to react with non-melanocytic tumors, such as angiomyolipomas, adrenocortical neoplasms, and other steroid cells tumors. A metastasis from an unknown primary is often encountered in cytology with both melanoma and renal cell carcinoma in the dfferential. However, very little data is available in regards to the proportion of renal cell carcinomas which stain with MART-1.

Design: Tissue microarrays were constructed from 52 archival formalin-fixed, paraffin-embedded renal cell carcinoma cases from the various anatomic locations. Cytology cell block material was also utilized. The tumor subtypes included (33 clear cell, 8 papillary, and 3 chromophobe renal cell carcinomas, 1 collecting duct carcinoma, and 7 oncocytomas). The sections were immunostained with MART-1 (DAKO Ab4 to A103) and semiquantitative evaluation (-, 1+, 2+, 3+) of the tumor cells was conducted.

Results: Four of 52 (7.55%) cases examined showed cytoplasmic immunoreactivity for MART-1, while 1 case showed 1+ nuclear staining. Three of the positive cases were convention clear cell renal cell carcinoma. One positive case was an oncocytoma and the case with nuclear immunoreactivity was also an oncocytoma. For a summary of our data, see *Table 1*.

MART-1 Staining Characteristics of Renal Cell Carcinomas

Tumor Type	1+ Intensity	2+ Intensity	3+ Intensity	Nuclear* reactivity	Total
Clear Cell	1	1	1	0	3 (n=33)
Papillary	0	0	0	0	0 (n=8)
Chromophobe	0	0	0	0	0 (n=3)
Collecting Duct	0	0	0	0	0 (n=1)
Oncocytoma	0	1	0	1	2 (n=7)
Total	1	2	1	1	5

**nuclear reactivity was not considered positive for this study*

Conclusions: This preliminary data suggest that although a small percentage, a surprising number of renal cell carcinoma cases show prominent expression of MART-1. This can be very important in cytology cases where the amount of tissue may limit the number of stains which can be performed. Our findings emphasize the need for use of adjunctive stains such as S100, HMB-45, RCC-Ma and Pax-2 in these setting of metastases of unknown primary.

331 Detecting High Grade Cervical Disease on ASC-H Cytology: Role of ProEx™ C and hr-HPV Testing

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Background: Detection of underlying high-grade cervical disease in cervical cytology specimens utilizing molecular markers known to be over-expressed in cervical carcinoma is the goal of much current research. ProEx™ C detects two such molecular markers, minichromosome maintenance protein 2 and topoisomerase II, which are associated with abnormal cell cycle regulation. This study was conducted to evaluate ProEx™ C as a marker for high grade cervical disease, as compared to high risk-HPV (hr-HPV) status and histologic diagnosis, in Pap tests with atypical squamous cells, cannot exclude HSIL (ASC-H) cytology.

Design: Cervical cytology specimens were collected in SurePath™ preservative and those with a diagnosis of ASC-H were included in the study. A second SurePath™ slide was prepared from the residual cellular material and stained using the ProEx™ C reagent (TriPath Imaging, Inc, Burlington, NC) prediluted with waterbath antigen retrieval, using Dako autostainer. SiHa cells were used as control cells and were optimized with SureDetect reagents. Nuclear staining of squamous cells was considered a positive result. hr-HPV testing using Digene's Hybrid Capture® 2 was performed on each case. A follow-up biopsy was performed within one month of the abnormal Pap test, for histologic diagnosis.

Results: A total of 100 patients with ASC-H diagnosis were part of the study. Age ranged from 21 to 79 years. The follow-up cervical biopsy results along with ProEx™ C and hr-HPV results are summarized in Tables 1 and 2. Cervical biopsy was used as the gold standard.

Table 1: Results of 100 ASC-H Pap Tests with follow-up cervical biopsy, ProEx™ C and hr-HPV testing.

Diagnostic Categories	Cervical Biopsy	ProEx™ C Positive Results	hr-HPV Positive Results
Negative for Dysplasia	10	2	4
CIN I	7	1	2
CIN II	62	61	48
CIN III	21	21	11

Table 2: Detection of CIN II+ disease in ASC-H Pap Tests, utilizing ProEx™ C and hr-HPV tests.

Test	Sensitivity	Specificity	PPV	NPV
ProEx™ C	98.79%	82.35%	96.47%	93.33%
hr-HPV	71.95%	64.70%	90.76%	32.35%

Conclusions: ProEx™ C staining, when compared to hr-HPV status in Pap tests with ASC-H, is a more sensitive and specific biomarker for detection of high grade cervical disease. It can be easily used in combination with routine Pap tests and hr-HPV tests to significantly decrease false positivity. ProEx™ C can locate abnormal cells at an intermediate magnification while screening and has a great potential in the future as a marker for detection of high grade disease in patients with ASC-H Cytology.

332 Longitudinal Follow-Up of Patients with a "Negative" Cervical Biopsy Following a Papanicolaou Test Interpretation of ASC-H

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Background: Approximately half of women with a papanicolaou test interpretation of ASC-H (*Atypical squamous cells, cannot exclude high-grade squamous intraepithelial lesion*) will have less than a high-grade dysplasia diagnosed on their follow-up cervical biopsy. In this study, we tested the null hypothesis that women with a negative cervical biopsy following a pap test interpretation of ASC-H (study group, SG) have no worse a spectrum of follow-up cytologic and histologic abnormalities of the cervix than a randomly selected control group of women whose pap tests were interpreted as *negative for intraepithelial lesion or malignancy* (NILM) during the same period (control group, CG).

Design: Clinicopathologic data on consecutive pap tests with ASC-H interpretations were reviewed, as was a randomly selected group comprised of NILM interpretations. Patients with previous ASC-H, HSIL or CIN 2-3 interpretations were excluded. The diagnoses in every follow-up sample in the control and study groups were tabulated. Both groups were then statistically compared regarding the diagnostic frequencies of each of the Bethesda 2001 categories and CIN grades (Fisher's Exact Test).

Results: Of the 122 patients with ASC-H interpretations *and* documented histologic follow-up, the *first* follow-up biopsy was negative for dysplasia in 20 (16%). 76 follow-up samples (70 pap tests, 6 biopsies) were obtained from these 20 patients. In the CG of 262 women with NILM interpretations, 641 follow-up samples (629 pap tests, 12 biopises) were obtained. Patients in the SG were significantly more likely than their CG counterparts to have a follow-up 1] cytologic/histologic abnormality (23/76 vs 76/641 respectively, p=0.00006), 2] ASC-US interpretation (13/76 vs 43/641, p=0.005) 3] CIN 1 diagnosis (4/76 vs 10/641, p=0.05), and 4] CIN 2-3 diagnosis (2/76 vs 2/641, p=0.05). The 2 groups did not significantly differ regarding the frequency of LSIL, HSIL, and ASC-H interpretations. If the analysis is restricted to one follow-up sample (the most severe) per patient, the SG patients were still more likely than those in the CG group to have a follow-up cytologic/histologic abnormality (12/20 vs 40/262, p=0.0002), ASC-US interpretation (6/20 vs 22/262, p=0.008) and follow-up CIN 2-3 diagnosis (2/20 vs 2/262, p=0.03).

Conclusions: Patients whose cervical biopsies are devoid of dysplasia following an ASC-H interpretation still require close surveillance and follow-up, as their risk of being diagnosed with follow-up cervical abnormalities is significantly above baseline.

333 Is a Second Pathologist's Review of ASC-H Useful in Reducing False Negative Diagnosis?

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Background: A diagnosis of a"typical squamous cells, cannot exclude HSIL (ASC-H)" on cervical cytology is usually followed by colposcopy and biopsy. The false negative rates on follow-up biopsies vary and is often due to immature squamous metaplasia or tubal metaplasia. The purpose of the study is to determine the impact a re-review of ASC-H by a second pathologist has on the true positives and false negative diagnoses.

Design: ASC-H cases were retrieved from the cytopathology files of an academic institution from 2003 to 2007. Only cases with follow-up biopsy were included in the analysis. All cases with follow-up biopsies were reviewed by a second pathologist without knowledge of the biopsy result. The re-review diagnoses included benign/reactive, low grade, ASC-H/HSIL. The rates of true positives and false negatives were compared.

Results: Out of 67,721 cases from the last 4.5 years, there were 135 cases with ASC-H, 86 (64%) of which had follow-up biopsies. The biopsy results included: Benign cases 48 (55.8%), CIN I cases 8 (9.3%) and CIN II/III cases 30 (34.88%). On second review of cervical cytology ASC-H was diagnosed in 48/86 (55.8%). Other diagnoses on second review included low grade and benign/reactive. The biopsy results showed a decrease in false negatives from 55.8% to 22.58%, however the true positives were also reduced from 34.88% to 25.58%.

Conclusions: Re-review of ASC-H diagnoses may reduce the rate of false negative diagnosis and prevent patients from having unnecessary colposcopic biopsy. However, this may also reduce the true positive rate. While reduction of unneeded colposcopic biopsy is desirable, however, since this is a screening test, the decrease in the true positive diagnoses may make a second pathologist's re-review of ASC-H cases unjustified.

Initial and second review of ASC-H with follow-up biopsy.

	Benign on Biopsy	CIN I on biopsy	CIN II/III on biopsy
ASC-H (Initial Review)	48 (55.8%)	8 (9.3%)	30 (34.88%)
ASC-H/HSIL on second review	22 (22.58%)	4 (4.65%)	22 (25.58%)
LSIL on second review	9 (10.46%)	2 (2.33%)	11 (8.13%)
Benign/reactive on second review	17 (19.77%)	2 (2.33%)	1 (1.16%)

334 Molecular Cytopathology – Use of ThinPrep Preparation for HER2 Tests in Breast Cancer: IHC, FISH, CISH and SISH

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Background: In our daily practice, HER2 tests by IHC and/or FISH have been done on formalin-fixed paraffin embedded (FFPE) sections. The well designed algorithm has been suggested by ASCO/CAP guideline which emphasize quality assurance and validation. In order to combine morphology, immunohistochemistry (IHC), fluorescence in situ hybridization (FISH), chromogenic in situ hybridization (CISH), silver in situ hybridization (SISH) and molecular studies (such as mutation of p53), we have attempted to apply ThinPrep preparation (Cytyc Cooporation). This study is aimed at to elucidate the feasibility of ThinPrep preparation for these morphological and molecular studies.

Design: ThinPrep preparation was collected from the resected specimens of breast cancer. ThinPrep cytology specimens were subjected to, in addition to Papanicolaou staining, IHC, FISH, CISH and SISH. IHC was done by anti-HER2 antibody (CB11), FISH (Abbott,Vysis), CISH (Invitrogen) and SISH (Ventana Medical Systems Inc.). Tissue sections were stained for IHC by HercepTest (DAKO). These procedures were done following the manufacturers' recommendations. From the pooled cells obtained by ThinPrep procedure, p53 mutation and FISH for EGFR (Abbott,Vysis) were performed in the selected cases of basal-like (triple negative) cancers.

Results: On ThinPrep cytology specimens, the cases with HER2 IHC 3+ showed amplification of HER2 gene by FISH. FISH on tissue sections and on ThinPrep cytology were comparable, i.e. 4.63 and 5.00 for IHC 3+ case, 1.79 and 1.76 for IHC 2+ case, 1.26 and 1.25 for IHC-case, respectively CISH on ThinPrep cytology correlated with FISH, i.e.presence of cluster formation in FISH amplified case (2.81), two gene copies in non-amplified cases. By SISH, detailed gene copies were discernible and HER2/CEP17 ratio was calculated,i.e.HER2/CEP17 was 7.2 for IHC 3+ case and 1.26 for IHC HER2-. In the cases of basal-like (triple negative) breast cancers, selected cases showed EGFR amplification (EGFR/CEP17 as 3.48). P53 point mutation was noted in Exon 8 (Cys→ Tyr or Arg→Pro) in particular cases.

Conclusions: Our results suggest that ThinPrep preparation of breast cancers could be applied for HER2 testing by molecular techniques such as FISH and SISH and further mutation analysis. It is expected to be suitable for ASCO/CAP guideline.

335 Hemosiderin Laden Macrophages and Hemosiderin in Follicular Epithelial Cells Strongly Discriminate between Benign Goiter and Follicular Neoplasm

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Background: Follicular neoplasms of thyroid have been differentiated from benign goiters based on abundant cellularity, microfollicular architecture, and scant colloid. Hemosiderin laden macrophages are commonly observed in benign goiter and papillary thyroid carcinoma, but we are unaware of the significance of these in follicular neoplasms. We tested the hypothesis that hemosiderin and macrophages virtually exclude the diagnosis of a follicular neoplasm.

Design: A random cohort of FNAs that had follow-up with histopathologically proven benign goiter (n=76), follicular adenomas (n=35), and follicular carcinomas (n=8) were studied. Thin preps and cell blocks of these cases were evaluated blindly at 40X by two independent observers for the presence of macrophages, macrophages with hemosiderin, and hemosiderin within follicular epithelial cells. The statistical significance of the findings was evaluated using the Chi-Square test with Yates' correction.

Results: Macrophages were observed in 62/76 (82%) of benign goiters, whereas only 5/43 (12%) of follicular neoplasms had any macrophages ($p<0.0001$). Of the five cases of follicular neoplasm with macrophages, the surgical pathology diagnosis was follicular adenoma with degenerative changes in 2 cases, one case showed a hurthle cell adenoma, and another showed a follicular adenoma associated with lymphocytic thyroiditis. There were no follicular carcinomas with macrophages ($p<0.1264$). Hemosiderin was present within the macrophages in 51/76 cases of goiter and in only 2 of 43 cases of follicular neoplasm ($p<0.0001$) which included a follicular adenoma with cystic degeneration. Hemosiderin was also present in follicular epithelial cells in 11/76 (15%) of benign goiters, whereas none of the follicular neoplasms showed intraepithelial hemosiderin ($p<0.0067$).

Table 1

Diagnosis	Macrophages	Hemosiderin in Macrophages	Hemosiderin in follicular cells
Benign goiter (n=76)	62[1]	51[2]	11[3]
Follicular adenoma (n=35)	5	2	0
Follicular carcinoma (n=8)	0	0	0

[1]$p<0.0001$; [2]$p<0.0439$; [3]$p<0.0067$

Conclusions: Macrophages, hemosiderin, and intraepithelial hemosiderin are powerful predictors of a benign lesion in thyroid cytology. Our results suggest that if papillary thyroid carcinoma and hurthle cell neoplasm can be excluded, the presence of macrophages and intracellular hemosiderin argues strongly against the diagnosis of follicular neoplasm.

336 Core-Biopsies Are Replacing FNAs in Most Organs

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Background: Needle core biopsies have replaced fine needle aspirations (FNA) as a diagnostic tool for breast and prostate and now threaten to take over in deep organs where FNA used to dominate. The study was performed to determine the magnitude and impact of this change.

Design: All specimens from liver, lung, kidney, and pancreas accessioned in the cytology lab in years 1997, 2002 and the first half of 2007 at the Beth Israel Deaconess Medical Center were culled. Biopsy type (touch imprint smear [TIS] from core-only, FNA or Both) was determined for each specimen. The diagnosis was categorized as benign, atypical, suspicious or positive. The proportion of each biopsy type was determined and compared for the years and organs studied. Percentage of diagnostic category for biopsy types was also compared.

Results: 665 specimens from the years 1997 (143), 2002 (265) and 2007 (257) were included in the study. These included lung (224), kidney (33), liver (172) and pancreas (236). Over the course of 10 years, percentage of TIS specimens increased from 3.5% to 25%, most prominent in the liver (10% in 1997 to 84% in 2007 as shown in the table). In contrast, percentage of FNA specimens reduced from 92% to 64%. FNA constituted 100% of the specimens from the pancreas in all years. Overall, the percentage of positive diagnosis was highest for TIS (68%) compared to both TIS-FNA (62%) and FNA (36%) alone. However, this trend varied according to year and organ. In 1997, the percentage of positive diagnosis was comparable with 60%, 55% and 83% for TIS, FNA and both, respectively. These changed to 66%, 23% and 50% respectively in 2007. Marked reduction in FNA specimens with a positive diagnosis was seen in liver from 67% to 17%.

Conclusions: At our institution, with the exception of pancreas, core biopsies are being increasingly used for deep organs especially when a malignancy is suspected. Consequently, cytopathologists frequently need to interpret TIS of core biopsies for specimen adequacy evaluation and immediate diagnosis. Since TIS has different morphology from FNA, this fact needs to be considered in training of residents and fellows for the future.

Year	1997	2002	2007	Total
Organ/type	N(%)	N(%)	N(%)	N(%)
LIVER				
FNA	43(86)	12(20)	6(10)	61(35)
TIS	5(10)	42(70)	52(84)	99(58)
BOTH	2(4)	6(10)	4(6)	12(7)
LUNG				
FNA	46(92)	80(73)	38(58)	164(73)
TIS	0(0)	7(7)	7(11)	14(6)
BOTH	4(8)	22(20)	20(31)	46(21)
KIDNEY				
FNA	1(100)	10(56)	5(36)	16(49)
TIS	0(0)	2(11)	5(36)	7(21)
BOTH	0(0)	6(33)	4(28)	10(30)

337 Atypical Glandular Cells (AGC): Do These Cases Need a Second Review?

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Background: The diagnosis of atypical glandular cells (AGC) usually requires clinical work-up and the patient often undergo colposcopy, endocervical biopsy/curettage, and endometrial biopsy/curettage. The false positive rates on follow-up biopsies vary, often due to tubal metaplasia, microglandular hyperplasia, endometrial stromal breakdown and polyps. This study was performed to determine the impact of second cytopathologist's opinion would have on false positive and true positive cases.

Design: The cytopathology files at our academic institution were searched over 4 ½ -yr period (2003-2007) for AGC cases with follow-up biopsy or curettage (cervical and/or endometrial). All cases with follow up biopsies were reviewed by a second pathologist and the diagnoses were divided into categories according to 2001 Bethesda System for reporting cervical cytology. The rates of true positives and false positives between the initial and second review were compared.

Results: There were 67,721 cervical cytology specimens for the said period with 121 cases with AGC diagnoses. Only 84/121 (69.4%) cases had follow-up surgical biopsy and/or curettage. 62/81 (76.5%) were false positives on initial diagnoses, while 23.5% are true positives. On review by a second pathologist, the false positive rate decreased to 30/81 (37%) while true positives slightly increased to 23/81 (28.4%).

Conclusions: Review by a second pathologists appeared to reduce the false positives and slightly increase true positives in AGC. Having a second pathologist review cases diagnosed as AGC may increase accuracy and minimize unnecessary procedures.

Initial diagnoses of AGC and follow up biopsy and/or curettage

	Benign on biopsy (n=62)	Premaligmant on biopsy (n=9)	Malignant on biopsy (n=13)
AGC, NOS (n=18)	15 (83.3%)	2 (11.1%)	1 (5.6%)
AGC, EC (n=32)	26 (81.25%)	5 (15.6%)	2 (3.13%)
AGC, EM (n=24)	17 (70.83%)	2 (8.33%)	5 (20.83%)
AGC, N (n=10	4 (40%)	0	6 (60%)

Premalinant = CINIII and complex atypical hyperplasia; malignant = endometrial carcinoma

Re-review of AGC diagnoses with follow-up biopsies

	Benign on biopsy (n=30)	Premalignant (n=10)	Malignant (n=13)
AGC, NOS (n=7)	4 (57.1%)	2 (28.6%)	1 (14.3%)
AGC, EC (n=14)	10 (71.43%)	3 (21.43%)	1 (7.14%)
AGC, EM (n=24)	16 (66.7%)	3 (12.5%)	5 (20.8%)
AGC, N (n=6)	0	0	6 (100%)
HSIL (n=2)	0	2 (100%)	0
NILM/reactive (n=31)	31 (100%)	0	0

Premalinant = CINIII and complex atypical hyperplasia; malignant = endometrial carcinoma

338 Cytological Evaluation of False Positive (FP) Cases of Papillary Thyroid Carcinoma (PTC) on Fine Needle Aspiration (FNA)

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Background: FP diagnosis of PTC in thyroid FNA is rare, but the presence of atypical features associated with thyroiditis and cystic changes may lead to over diagnosis. This study evaluates cytological features associated with FP diagnosis of PTC to explore how to avoid diagnostic pitfalls.

Design: Fifteen FP thyroid FNAs were collected from our archives from 2000 to 2007. Another 15 true positive (TP) cases were randomly selected as control from the same time period. All cases were blindly reviewed by 4 participating cytopathologists. Seventeen cytological features were scored as absence (0) to present (scored 1-3: seen at high (1), medium (2), and low (3) magnification and a diagnosis rendered. All the feature scores were tabulated and analyzed.

Results: All cases had both DQ and Pap stained conventional slides and some also had ThinPrep and cell block slides. Patients were mostly female (23 female, 7 male) with an average age of 49 (ranging from 10-82, with TP group older than FP group (54 vs. 44). Among the 17 cytological features, the following 8 were scored differently in TP vs. FP groups: presence of macrophages (1.3 vs. 0.5), giant cells (0.7 vs. 0.3), squamous metaplasia (0.7 vs 0.2), Psammoma body (0.3 vs. 0.02), papillae (1.1 vs. 0.25), nuclear clearing (1.5 vs. 0.8), groove (1.7 vs. 0.85), and nuclear inclusion (0.86 vs. 0.34). Only squamous metaplasia, papillae, and nuclear inclusion were scored differently by all reviewers while histocytes, Psammoma body, giant cells, and nuclear clearing were scored differently by 3 of 4 reviewers. Surprisingly, the presence of histocytes, commonly known as a feature associated with benign condition, was seen more in TP group. Of the 15 FP cases, 7 were called PTC by at least one pathologist at review (2 by all 4 reviewers, 1 by 3, and 4 by 1 to 2 pathologists). Most of them had several nuclear features such as groove and nuclear clearing that are associated with PTC, thus leading to FP diagnosis even at the second review during our study. Interestingly, 7 of the 15 TP were missed on review by at least one pathologist (one was missed by all, four by 3, 3 by at least one pathologist), all but one lack of nuclear inclusion.

Conclusions: Many cytological features associated with PTC are not interpreted with a high level of reproducibility. Only three, one nuclear, features are consistently scored differently between TP and FP groups. Cases that were missed on review are being subjected to a more vigorous statistical analysis to identify features associated with mis-diagnosis.

339 Glypican-3 Protein Expression in Metastatic Melanoma: An Immunocytochemistry Study in Fine Needle Aspirate Archival Samples

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Background: The incidence of melanoma is increasing worldwide. Fine-needle aspiration (FNA) is critical in documenting recurrent and metastatic disease in established cases. The inherent potential of metastatic melanoma (MM) to mimic epithelial tumors presents a diagnostic dilemma. In FNA specimens, the distinction between primary hepatocellular carcinoma (HCC) and MM is a frequent challenge. Glypican-3 (GPC3), a heparan sulfate proteoglycan, has been proven to be a highly sensitive and specific marker for HCC. Serum GPC3 has been suggested by some authors as being expressed in 40% of primary melanomas. No tissue studies have been done to assess GPC3 expression in MM. To our knowledge, in this study, GPC3 protein expression is explored for the first time in FNAs from MM cases.

Design: Archival direct smear and Cytolyt-fixed (Cytyc, Boxboro, MA) material from 60 patients with confirmed FNA-diagnosed MM were retrieved. Following blockage of endogenous peroxidase and protein, antigen retrieval was performed. Primary antibody GPC3 (BioMosaics Inc, Burlington, VT), and secondary antibody Mouse Envision Polymer (DakoCytomation, Dako Corporation, USA) were used, followed by DAB chromogen (DakoCytomation). AEC chromagen (Dako cytomation) was used for pigmented cases. The slides were counterstained with hematoxylin, and examined by 4 observers. Moderate or strong cytoplasmic staining with membranous accentuation was considered positive. Weak or absent cytoplasmic staining were considered negative. FNA specimens from HCC cases and benign hepatocytes were used as positive and negative controls respectively.

Results: All HCC controls stained appropriately positive, and all benign hepatocyte controls were appropriately negative. All FNA slides from MM cases were negative (0/60) for GPC3. The exact 95% Clopper-Pearson confidence interval is 0.0% - 5.96%.

Conclusions: In this study, 0% of MM cases in archival FNAs were positive for GPC3. Our data supports the potentially significant diagnostic utility of GPC3 as a reliable tool in differentiating HCC from MM in FNA material.

340 Anal Pap Tests: Correlation of Cytology, HPV Results and Anal Biopsy

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Background: Anal Pap tests are used for screening patients at risk for developing anal intraepithelial neoplasia (AIN). These tests are reported using the Bethesda system terminology, used for cervical cytology. Specific Pap screening guidelines and optimal use of high risk HPV (hrHPV) testing have not been established. There are few studies reporting the correlation of anal Pap results with hrHPV status and anal biopsy.

Design: Patients (pts) with anal Paps during a 4 yr period from 2003 and 2007 were identified using a computerized search of the AP archives. All were manually screened ThinPrep Paps (Cytyc Corporation, Marlborough, MA). The cytologic diagnosis in each case was recorded. In the majority, HPV testing was performed using the Digene Hybrid Capture II method. HPV results and anal biopsy were recorded.

Results: A total of 111 anal Pap results were available from 97 pts (84 males, mean age 43). HPV testing was performed in 83 pts and anal biopsies in 36 pts. The distribution of anal Pap diagnoses and high risk HPV status is summarized in Table 1.

Table 1: Distribution of Anal Pap Results and HPV Status

Anal Pap Dx	Total Number	# Positive HPV/# Tested
NILM	71	19/56
ASCUS	20	14/18
LSIL	12	9/11
HSIL+	5	4/5

Anal Pap results and anal biopsy results are summarized in Table 2.

Table 2: Anal Pap Results with Surgical Follow Up

	Negative for Dysplasia	Condyloma/AIN 1	≥ AIN 2
NILM	3	9	4
ASCUS	0	4	3
LSIL	0	3	7
HSIL+	0	0	3

Three Paps were interpreted as unsatisfactory. 16 of 17 pts with AIN 2 or worse had HPV testing performed; 15 (93.8%) were HPV positive and 1 pt with AIN2 was HPV negative.

Conclusions: The majority of patients with ASCUS by anal Pap are HPV positive (78%). All ASCUS patients with biopsies had a diagnosis of AIN1 or worse. All but 1 pt with AIN 2 or worse were HPV positive. An anal Pap test alone would have missed 4 of 17 (23.5%) AIN2 or greater. Additional study is needed to delineate the role of anal Pap and/or HPV testing in patients at risk for AIN.

341 Hypocellular Pancreatic Cyst Aspirates – What Are We Missing?

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Background: Endoscopic ultrasound (EUS) guided fine needle aspirations (FNAs) of pancreatic cysts often yield few or no epithelial cells with little or no atypia. Since many cysts are incidental findings and/or have a non-specific radiologic imaging, the question arises how to follow-up these lesions. The aim of this study was to obtain follow-up on these types of cystic lesions thereby gaining insight into their clinical significance.

Design: Hypocellular EUS guided pancreatic cyst FNAs consisting of few or no epithelial cells with minimal or no atypia were selected from our files (2001-2006). The patient's age, sex and presenting complaints were recorded. Cyst characteristics including size, location, multiplicity and complexity were noted as per the radiology report. Subsequent clinical history, radiological findings and/or pathology tissue reports were obtained from the patient's medical record.

Results: 38 patients (19 males/20 females), ages 41-89 were selected. 20/38 patients had symptoms referable to the pancreatobiliary system. Cyst size varied from 0.9cm - 16cm with locations from the head (20), neck (3), body (3) and tail (9). There were 19 complex cysts, 8 simple cysts, 3 multiple cysts and 9 cases with non-specific morphology or not classified. Four patients were lost to follow-up (one with probable carcinoma, radiologically) and one died due to an unrelated cause. For the other patients, the follow-up ranged from 0-66 months (average 17.6). Of the 20 head cysts histological follow-up was available in 3: 1 IPMN with low-grade dysplasia (complex cyst), 1 serous cystadenoma (septated with calcification) and 1 metastatic granulosa cell tumor (septated). Two cysts resolved on follow-up CT. Of the 3 body cysts, histological follow-up was available in two: 1 mucinous cystadenoma (septated) and 1 IPMN (complex). None of the neck/tail cysts had histological follow-up, however, one (previous history of GIST, s/p distal pancreatectomy) subsequently developed a complex cyst (6.7cm) that was felt to be a post-operative abscess. The remainder of the patients were well without symptoms demonstrating a stable or reduced cyst size.

Conclusions: Most hypocellular cysts exhibit a benign course. Patients can be followed safely with repeat CT scan to monitor cyst characteristics +/- aspiration.Complex / multiple cysts (head or body) are at the greatest risk for a significant lesion.

342 Follow-Up Results of Reflex hrHPV Testing in Vaginal ThinPrep Specimens with a Diagnosis of ASC-US

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Background: The use of reflex high risk human papillomavirus (hrHPV) testing as a triage method for cervical smears with an interpretation of atypical squamous cells of undetermined significance (ASC-US) is well established. Very little has been reported regarding the utility of this approach in vaginal smears in women with prior hysterectomy, although providers often request hrHPV testing in this situation. The

purpose of this study was to evaluate results of hrHPV testing in women with vaginal smears interpreted as ASC-US.
Design: Follow-up information, including results of hrHPV testing, was sought for all vaginal smears reported as ASC-US from our cytology laboratory during the calendar year 2005. The laboratory exclusively utilized the ThinPrep Pap Test (Cytyc, Marlborough, MA) and Digene Hybrid Capture 2 hrHPV testing (Digene, Gaithersburg, MD) and accessioned approximately 72,700 Pap Tests. Computerized laboratory records were examined for the results of reflex hrHPV testing on these specimens, as well as any subsequent cytologic or surgical pathology results.
Results: Our laboratory accessioned 5430 vaginal specimens during the time studied, which constituted 7.5% of all gynecologic cytology accessions. Of these, 161 (2.6%) were interpreted as ASC-US. Reflex hrHPV testing was requested in 96, with sufficient residual material available in 80 (83.3%). hrHPV was detected in 22 (27.5%). Follow up (F/U) results were available for 51 of these 80, including 15 hrHPV detected (68%) and 36 hrHPV not detected (62%). Results are presented in Table 1; no high grade lesions were identified. F/U of hrHPV detected specimens showed low grade lesions (LSIL) in 40%, while LSIL was present in 8.3% on F/U of those with hrHPV not detected (p=0.023, Fisher's exact test).

Follow-Up Results

	LSIL	ASC-US	Negative	Total
HPV +	6 (40%)	5 (33.3%)	4 (26.7%)	15
HPV -	3 (8.3%)	3 (8.3%)	30 (83.3%)	36
Totals	9	8	34	51

Conclusions: Rates of detection of squamous abnormalities in women with ASC-US on vaginal preparations with hrHPV detected are higher than in those without hrHPV. These findings suggest that clinical follow-up is needed for women with hrHPV detected. hrHPV testing may be clinically useful as a method of triage for women with ASC-US vaginal smears.

343 Cytological Grading of IPMN: Does It Predict Histological Grade?

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Background: Cytological grading of intraductal papillary mucinous neoplasms (IPMN) on fine needle aspiration (FNA) samples has been proposed as a means to determine which patients need surgical intervention due to the likelihood of disease progression. This study evaluates the accuracy and limitations of FNA for predicting the histological grade of IPMN.
Design: Forty-eight FNA samples obtained from 37 patients who had a pancreatic resection with a diagnosis of IPMN were reviewed Six patients had multicentric disease (each site aspirated), one patient had one cyst aspirated twice and one patient had one cyst aspirated thrice. The cytology slides were reviewed for specific criteria in the following categories: background, architecture, cellularity, cytoplasm and nuclear. Each FNA sample was classified as IPMN with low grade dysplasia (LGD), moderate dysplasia (MD), or high grade dysplasia with or without invasive carcinoma (HGD/INV) based on sets of criteria established for each category prior to review. The samples were graded according to the feature imparting the highest grade. Samples were classified as nondiagnostic (ND) if they lacked recognizable neoplastic epithelium. The resection specimens were reviewed to verify the grade. The cytological grade was correlated with the histological grade at the biopsy site.
Results: Results are summarized in Table 1.

Comparison of Cytology to Histology for Each Cyst

	Histology				
Cytology	LGD	MD	HGD/INV	ND	Cytology Totals
LGD	13	0	2	0	15
MD	0	10	0	0	10
HGD/INV	1	2	12	0	15
ND	1	5	2	0	8
Histology totals	15	17	16	0	48

Statistical analysis using Fisher exact test showed a strong significant association between cytology and histology grading (p value <0.00001). The accuracy rate is 87.5% (excluding ND). Inherent sampling difficulties accounted for most of the missed MD samples. Disagreements in the LGD and HGD/INV categories were due to errors in recognizing subtle features. A key observation in the resections was the significant heterogeneity of the IPMN lining, in which ducts adjacent to the aspirated sites harbored a different grade of dysplasia. All six cases with multicentric disease were heterogenous and only two IPMN were homogenously composed of LGD.
Conclusions: FNA can predict histological grade of dysplasia in IPMN but is limited by heterogeneity of the lining epithelium and sampling difficulties. Cytological grading of IPMN would benefit from further diagnostic experience and molecular studies.

344 Preliminary Assessment of Fine Needle Aspiration Specimens by Telepathology: Validation for Use in Pathology Resident and Faculty Consultations

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Background: Telepathology (TP) has been used in settings where the distance between the pathologist and the diagnostic material is an obstacle to timely diagnosis, including underserved hospitals, frozen section diagnoses, and second opinions. Performance of fine needle aspiration (FNA) by radiologists and pathology residents at locations distant from pathology faculty can limit timely assessment of adequacy and preliminary diagnoses. The use of TP could overcome this limitation and allow for the acquisition of additional material, when needed. In this study, we evaluate the use of TP for consultation between residents and faculty in the preliminary assessment of FNA's.
Design: We tested a TP system that transmits dynamic microscopic images from a pathology resident microscope to a distant faculty computer. Two residents with varying experience (1 month and 3 months of cytology) screened and showed a total of 100 consecutive FNA cases to two faculty pathologists via TP. A speaker telephone was used for verbal communication. Resident and faculty interactions were timed, and the number of slides was recorded for each case. Diagnostic agreement for the TP assessment was compared with the original preliminary assessment and final diagnosis rendered by other faculty.
Results: We found diagnostic concordance rate of 97% and a diagnostic accuracy rate of 99%. Of the three cases with discordant results, one was considered clinically important and two were deemed an improvement upon the original assessment. For the clinically important case, the level of experience of the resident, the degree of cellular atypia, and the image quality all likely contributed to the misinterpretation (overcall of malignancy) by TP. For the other two cases, a concurrent core biopsy and a repeat FNA, respectively, agreed with the TP diagnosis of neoplasia. Total time for screening and faculty consultation ranged from 1 minute and 12 seconds to 34:11 (mean = 10:27). Those cases requiring longer time had numerous passes (overall range 1-22 slides, mean = 6.5).
Conclusions: Our TP system, with cooperation of residents and staff pathologists, may be an efficient and accurate method for the initial assessment and preliminary diagnosis of FNA specimens. Since our model of TP is extremely dependent on the skill and experience of the microscope operator, we recommend its use with more senior residents or fellows. Similar pitfalls inherent to traditional microscopic cytology apply to TP.

345 Comparison of Prediction Results from Single Gene Expression and Multigene Signatures in Matched Clinical Fine-Needle Aspiration (FNA) and Core Biopsy (CBX) Samples from Patients with Breast Cancer

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Background: We have reported that mRNA expression levels of estrogen receptor (ER) and HER2 obtained from microarrays of FNA or tissue samples can determine ER and HER2 status (Lancet Oncology 2007;8:203). We also derived a multigene signature to predict response to chemotherapy (CT) from FNAs (J Clin Oncol 2006;24:4236) and derived a signature for endocrine therapy (SET) from tumor tissues (ASCO 2007). The purpose of this study was to compare predictive genomic tests in matched pairs of FNA and CBX samples obtained in a clinic setting.
Design: Matched FNA and CBX breast cancer samples were obtained from 18 patients prior to neoadjuvant chemotherapy. RNA was prepared for gene expression profiling with Affymetrix (Santa Clara, CA) U133 microarrays. We measured the expression of ER mRNA (ESR1, probe set 205225), HER2 mRNA (ERBB2, probe set 216836), the CT signature (30 probe sets), and the SET signature (200 probe sets). Expression differences (t-test and Wilcoxon test) and Spearman correlation coefficients were compared in the FNA and CBX samples (2-tail, 95% significance). Area under the receiver operating characteristics curve (AUC) was used to measure predictive accuracy in FNA and CBX samples compared to standard pathologic ER status for ER mRNA and SET, HER2 status for HER2 mRNA, and pathologic response for CT.
Results: There was a nonsignificant trend toward higher expression of single genes and multigene signatures in FNAs, compared to matched CBX samples. Mean HER2 mRNA levels were higher in FNAs, but the rank order Wilcoxon test was not significant (p=0.07). FNA and CBX results were significantly correlated, except for SET. Predictive accuracy was high for both sample types, but the AUC values from FNAs were higher.

	ER	HER2	CT	SET
Mean expression: FNA, CBX, p value	1078, 910, NS	2286, 1505, p=0.04	-2.32, -8.41, NS	1.71, 1.37, NS
Correlation FNA vs CBX, p value	0.65, p=0.004	0.73, p=0.006	0.63, p=0.005	0.27, NS
Accuracy FNA (AUC)	0.93	0.98	0.80	0.91
Accuracy CBX (AUC)	0.91	0.83	0.57	0.83

Conclusions: Levels of ER mRNA, HER2 mRNA, and multigene predictors were similar in matched FNA and CBX samples. FNAs tend to provide slightly higher gene expression levels than CBXs, and to be more accurate when compared to standard pathologic tests or patient outcomes.

346 Value of P63 and CK5/6 Panel in Distinguishing Squamous Cell Carcinoma from Adenocarcinoma in Lung Fine-Needle Aspiration (FNA) Specimens

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Background: The current FDA approved standard of care for non-small cell lung cancer is carboplastin/taxol/Avastin based upon an impressive survival benefit relative to chemotherapy alone. However, only patients with non-squamous cell carcinoma can receive Avastin because of a 30% mortality rate due to fatal hemoptysis observed in patients with squamous histology. The purpose of this study was to evaluate the role of cytomorhology and immunostains in differentiating squamous (MSCC) vs. adenocarcinoma (MAD) in lung FNA specimens.
Design: The case cohort included 51 cases of non-small cell lung carcinoma FNA specimens with surgical pathology follow-up. All FNA specimens were reviewed independently by a panel of cytopathologists to differentiate between squamous (MSCC) and adenocarcinoma (MAD) on the basis of cytomorphology. The cell block material was available in 20 cases (10 MAD and 10 MSCC) to perform immunostains for TTF1, CK7, CK20, P63, and CK5/6.
Results: On surgical resection 35/51 (69%) were diagnosed as MAD, and 16/51 (31%) as MSCC. The overall number of cases classified correctly by all participants on the basis of cytomorphology alone was 69% for MAD and 58% for MSCC (combined accuracy 65%). By immunostaining, 13/20 (65%) cases were TTF1 positive, 16/20 (80%) were CK7 positive, while only one case (5%) showed focal CK20 positivity.

The majority of the TTF1 negative cases (60%) were MSCC. Both P63 and CK5/6 expression was seen in 8/10 (80%) MSCC, however, none of the 10 MAD cases showed this dual expression.

Conclusions: Cytomorphology alone may not be able to further stratify all cases of non-small cell lung carcinoma into MAD and MSCC in FNA specimens. The immunopanel of TTF-1, CK7, P63 and CK5/6 appears to be useful in differentiating MSCC from MAD, which can be beneficial for patient management.

347 Fine Needle Aspiration of Soft Tissue and Bone Lesions Avoids the Need for Confirmatory Biopsy and Repeat Immunohistochemistry

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Background: Fine needle aspiration (FNA) of soft tissue and bone lesions (STBL) is viewed primarily as a triage method prior to an invasive second stage procedure (open biopsy or frozen section at the time of excision). This study evaluated whether FNA of STBL can provide sufficient information for definitive treatment without a second stage procedure. Because immunohistochemical (IHC) stains provide important information when evaluating STBL the accuracy of IHC stains performed on FNA material was compared to IHC stains on excision material.

Design: All FNA cases of STBL performed at our institution between January 1, 2003 and December 31, 2006 were retrieved. Consult cases and mesenchymal lesions of solid organs were excluded. For each case the following were recorded: primary or recurrent STBL, IHC stains on FNA, open biopsy, frozen section during definitive excision, excision diagnosis, and IHC stains on excision.

Results: A total of 161 cases were identified comprised of 138 primary lesions (85.7%) and 23 recurrent lesions (14.3%) (see table). Only 19 cases (11.8%) had a second stage procedure between FNA and definitive excision. Specific cytologic diagnoses were made in 129 cases (80.1%) and descriptive diagnoses in 32 (19.9%). 79 primary cases and 14 recurrent cases had surgical follow-up with concordance rates of 97.5% and 100% respectively (overall concordance rate of 97.8%). The 59 primary STBL with no surgical follow-up were: 37 lipomas, 8 specific benign lesions, 8 descriptive lesions, and 6 specific malignant lesions. 37 FNAs used IHC (23.0%). Of these, 15 cases (9.3%) had IHC stains repeated on surgical follow-up (48 repeated antibodies, ranging 1-6/case, mean 2.7). Only 5 antibodies were discrepant due to focal positivity noted on excision material (negative cytology result, 4 cases) and background staining (1 unstained smear).

	Cases with surgical follow-up (% of total cases)		Cases with no surgical follow-up (% of total cases)
	Second stage procedures	No second stage procedures	
Primary (138)	19 (11.8%)	60 (37.2%)	59 (36.6%)
Recurrent (23)	0	14 (8.7%)	9 (5.7%)
Total (161)	19 (11.8%)	74 (45.9%)	68 (42.3%)

Conclusions: Our experience is that FNA of primary and recurrent STBL in conjunction with IHC provides sufficient information to the clinician to proceed with definitive treatment without the need for subsequent open biopsy or confirmatory frozen section at the time of excision. IHC stains performed on FNA material are accurate and do not need to be repeated on the excision material.

348 Evaluation of Disseminated Tumor Cells in Bone Marrow in Operable Breast Cancer

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Background: There is a renewed interest currently in the evaluation of disseminated tumor cells (DTC) in bone marrow (BM) because of their potential impact on prognosis,survival and overall management of patients with breast cancer.We report here the evaluation of DTCs in BM aspirates by conventional cytology and cytokeratin (CK) immunostaining of specimens enriched for tumor cells.

Design: BM aspirations were performed from bilateral iliac crests in a IRB approved protocol in 75 patients with operable invasive breast cancer before primary breast surgery. The aspirates were subjected to Ficoll Hypaque density gradient centrifugation. Ten cytospin slides were prepared; 1 slide for Papanicalaou staining and remaining for immunocytochemistry (ICC) using CK cocktail of AE1/AE3,CAM5.2,MNF116. Positive staining in morphologically atypical cells was regarded as a positive result. Presence of such cells (irrespective of their number) was correlated with primary tumor characteristics and lymph node status by Chi square statistical analysis.

Results: Seventy five (75) patients were enrolled in this prospective study who were staged as T1=25, T2=33, T3=7, T4=10; ductal in type in 55 and lobular in 19; Lymphovascular invasion (LVI) was noted in 24/73 cases; Sentinel lymph nodes and/or axillary dissection were positive in 35 cases. Primary breast tumor was positive for estrogen receptor in 48/75, progesterone receptor in 38/75, HER2/neu by FISH in 11/75 cases. Cytological evaluation of the Pap stained slide showed few clusters of degenerated possibly tumor cells in one case only. ICC staining for CK revealed predominantly scattered (13) and rare clusters (4) of positive cells with atypical morphology, compatible with tumor cells in 17 of the 75 cases (23 %). Six of these cases were staged as T1(24%), seven as T2(21%), two as T3(28%), and two as T4(20%). Chi square analysis did not reveal any correlation between primary tumor characteristics including tumor size, tumor type, LVI, hormone receptor and HER2 status metastatic carcinoma in axillary lymph nodes and the presence of DTCs in BM aspirates.

Conclusions: 1) DTCs occurred in 23 % of operable breast cancers predominantly as rare single cells as revealed by CK ICC of BM aspirates enriched for tumor cells. 2) Conventional cytological examination was not found to be useful for detecting DTCs. 3) The lack of correlation between the occurrence of DTCs and lymph node metastasis indicates different routes of dissemination of the tumor cells to these two sites. 4) No predictors for the occurrence of DTCs in BM was found in this study.

349 Utility of Glypican-3, Alpha-Fetoprotein, and HepPar-1 as a Panel for Detecting Hepatocellular Carcinoma in Fine Needle Aspiration Biopsies

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Background: The cytological diagnosis of hepatocellular carcinoma (HCC) in fine needle aspiration biopsies (FNAB) may be difficult. Cytomorphology alone can be insufficient to discern well- differentiated HCC from benign lesions such as regenerative nodules, or poorly-differentiated HCC from other malignancies. Traditional markers such as alpha-fetoprotein (AFP) or HepPar-1 lack the desired sensitivity or specificity. Previous studies have shown that Glypican-3 (GPC-3) is a promising marker for the diagnosis of HCC in surgical specimens. We examine the utility of GPC-3 specifically for diagnosing HCC in hepatic FNAB specimens, when used as part of a panel with AFP and HepPar-1.

Design: Forty-one cases were retrieved from the cytopathology archives at the University of Pittsburgh Medical Center-Shadyside Hospital. Based on the cytopathologic diagnosis, the cases were segregated into three groups: 1) HCC, 2) Poorly differentiated carcinoma (PDCa), and 3) Benign. The benign group included cases diagnosed as cirrhosis, regenerative nodules, and benign or reactive hepatocytes. The FNAB smear slides (Diff-Quik and Papanicolaou) and cell-block sections (H&E) were reviewed. The immunostains for GPC-3, AFP, and HepPar-1 were performed on the cell-block sections with approriate controls. The results of immunostains were considered positive if diffuse or focal reactivity was present, and negative if there was no or rare reactivity.

Results:

Expression of glypican-3, alpha-fetoprotein, HepPar-1

		GPC-3		AFP		HepPar-1	
	Cases (n)	Positive	Negative	Positive	Negative	Positive	Negative
HCC	18	13 (72%)	5 (28%)	7 (39%)	11 (61%)	15 (83%)	3 (17%)
PDCa	12	6 (50%)	6 (50%)	3 (25%)	9 (75%)	4 (33%)	8 (67%)
Benign	11	3 (27%)	8 (73%)	0	11 (100%)	11 (100%)	0

Conclusions: GPC-3 immunoreactivity was demonstrated in the majority of the cases in the HCC group (72%), including three HepPar-1 negative cases. Two of the five GPC-3 negative cases were AFP positive. One-half of the PDCa cases were GPC-3 positive, and interestingly, five of the six GPC-3 positive cases appeared to have cytomorphologic features suggestive of HCC. In the benign group, GPC-3 positivity was seen in three cases. The follow-up core biopsy or resection specimens for these three cases showed a well-differentiated HCC. Thus, GPC-3 may be a useful tool in the cytologic diagnosis of HCC, particularly when used in combination with AFP and HepPar-1.

350 Incidence of Polyomavirus Infection in Urine Specimens from Patients with Positive FISH Results

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Background: Polyomavirus infected cells in the urine may be a cause of false positive result in routine urine cytology. A previous study showed that 12.5% of urine specimens with polyomavirus infection were positive for fluorescence in situ hybridization (FISH) raising the concern that polyomavirus infection may cause false positive results in FISH. This study attempted to determine the incidence of polyomavirus infection in cytology specimen from patients with positive FISH results.

Design: Urine samples were collected from patients in multiple different urological centers. The sample was split and urine cytology and fluorescence in situ hybridization for the detection of bladder cancer were performed. Urine cytologies were stained using standard Papanicolaou stain. FISH was performed using chromosome enumeration probes 3, 7, 17, and locus-specific probe 9p21 (p16 gene). FISH slides were screened by a cytotechnologist to determine if aneuploidy was present. Corresponding cytologies from FISH positive patients were screened for evidence of polyomavirus infection.

Results: Out of 158 patients with positive FISH results polyomavirus infection was present in 12 patients after cytologic evaluation. This corresponded to an incidence of polyomavirus infection in 7.6 % of patients that had positive FISH results.

Conclusions: Polyomavirus was present in a small but significant percentage of cytologies that were positive for aneuploidy by FISH. This study does not validate the suggestion that polyomavirus infection can interfere with the result of FISH evaluation, however, it does show a higher than expected incidence of polyomavirus in FISH positive patients. Further research should be done to determine if polyomavirus infection can affect the results of FISH for the detection of bladder cancer.

351 Low-Grade Neuroendocrine Neoplasms of the Lung on Fine Needle Aspiration: Where Do We Make Errors in Interpretation?

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Background: Neuroendocrine (NE) neoplasms of the lung, i.e., carcinoid tumor (CT), atypical carcinoid tumor (ACT) and small cell carcinoma (SCC) may lead to diagnostic errors on fine needle aspiration (FNA) due to their often overlapping morphologic characteristics. The cytopathologic distinction of CT and ACT from SCC is clinically relevant for important therapeutic and prognostic implications.

Design: The cytopathology archives of a major teaching hospital revealed 57 cases of CT and ACT diagnosed on FNA in a period of 18 years (1990-2007). Of these, 51 cases had appropriate histologic follow-up. The clinicoradiologic, cytologic, histologic and immunoperoxidase (IPOX) staining characteristics were reviewed and correlated. Cytologic material was obtained by transthoracic CT-guidance or tranbronchial FNA of the lung. The smears were stained with Diff Quik and Papanicolaou stains, and the cell block sections from needle rinses were stained with H&E stain.

Results: A total of five (9.8%) cases of low-grade NE neoplasm were over diagnosed on FNA as SCC (n=3) or poorly differentiated carcinoma, NOS (n=2). A retrospective review of the cytologic material revealed that the likely source of misdiagnoses included; unusually high cellularity, cellular pleomorphism with oval to spindled nuclei, nuclear

hyperchromasia, finely granular chromatin, nuclear molding and occasional pyknotic nuclei. In addition, artifacts due to air-drying of the smears and poor cellular preservation were noticeable in 4/5 cases. None of these five cases showed karyorrhexis and/or mitoses (classic features of SCC). Three of the five cases on follow-up biopsy/resection were CT, whereas two turned out to be ACT.

Conclusions: Over diagnosis of CT/ACT as SCC on FNA is rare (10%).The key feature for an accurate distinction of CT/ACT from SCC is lack of cellular kayorrhexis and mitoses. All other cytomorphologic features observed on FNA (as listed above) can be seen in both tumor types (i.e., CT/ACT and SCC) and should not be solely relied upon for cytopathologic diagnosis. IPOX stains play a limited role in FNA due to an often small sample size and overlapping staining characteristics. It is prudent to be extremely careful when rendering diagnosis on sub optimally prepared and air-dried smears of CT/ACT. Both CT and ACT may potentially lead to an over diagnosis of SCC.

352 Histone Modification Characteristic of Papillary Thyroid Carcinomas Is Directly Induced by RET and TRK, and Is Associated with Nuclear Lamina Irregularity

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Background: Our previous studies identified a loss of monomethylation of histone H3 at lysine 4 (H3K4m) in samples of papillary thyroid carcinoma (PTC). Since RET and TRK tyrosine kinases are sufficient to cause the heterochromatin dispersal and nuclear lamina irregularity characteristic of PTC, we tested whether RET or TRK expression is sufficient to cause loss of H3K4m, and whether this histone modification relates to nuclear lamina irregularity.

Design: Primary cultures of human thyroid epithelium were infected with an adenoviral construct bearing RET/PTC1 and a red fluorescent protein reporter, fixed at 48 hours, immunostained for various histone modifications, and scored for expression levels of modified histones in relation to RET expression and nuclear lamina irregularity. Western blotting compared expression levels between primary cultures retrovirally transduced to express either TRK/PTC (TRK T3) or a catalytically inactive TRK/PTC (TRK T3 ABN).

Results: Within 48 hours, RET/PTC1 induces loss of H3K4m ($p<.001$ by McNemar's test) and H3K27m ($p<.001$) without affecting levels of H3K9m (P=0.43), H3 acetylation on K9 or K14 (P=0.08), or H2AX phosphorylation. The loss of H3K4m was significantly greater in cells with irregular nuclear lamina contour compared to cells that retained regular nuclear lamina contour ($p<.001$ by Fisher's exact test) whereas loss of H3K27m was not statistically different between cells with regular and irregular nuclear lamina. TRK T3 caused 4 fold loss of H3K4m compared to catalytically inactive TRK T3 ABN, without altering the expression levels of di-methylated H3K4 and without affecting global expression levels of the H3K4 demethylase LSD1.

Conclusions: Global loss of H3K4m in PTC is mediated by two oncogenes that cause the nuclear lamina irregularity and chromatin dispersal characteristic of PTC, and this histone modification segregates with the phenotype of nuclear lamina irregularity. We propose that loss of H3K4m is an important early event in transformation with a direct relation to the nuclear structural changes diagnostic of papillary thyroid carcinoma.

353 Fine Needle Aspiration of Thyroid Micronodules

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Background: The widespread use of ultrasound in evaluation of thyroid nodules has resulted in an increase in fine needle aspiration (FNA) of thyroid nodules less than 1.0 cm in size (micronodules). Current practice guidelines recommend FNA for thyroid nodules measuring ≥1.0 cm in size; those less than 1.0 cm should be aspirated only if the nodule is radiographically suspicious or if there is a clinical concern. Our objective was to review our experience with FNA of thyroid to determine if there is a difference in the incidence of carcinoma detected by FNA in micronodules in comparison to nodules measuring ≥1.0 cm.

Design: The departmental computer system was searched for thyroid FNAs from 1/1/2002 to 6/31/2006. Nodule size and cytologic diagnoses were compiled. The cytologic diagnoses were compared for nodules <1.0 cm to those ≥1.0 cm.

Results: There were 2335 thyroid nodules from 1544 patients (264 males, 1280 females with a 1:5 male/female ratio) after excluding 105 cases due to unsatisfactory sampling, and 1043 due to insufficient clinical information (nodule size not reported). The average age of patients was 54 ± 2.1 ((mean ± std) years. The average nodule size in nodules ≥1.0 cm was 2.1 ± 1.1cm (mean ± std) (range 1.0-9.0 cm). The average nodule size in micronodules was 0.8 ± 0.1 cm (mean ± std) (range 0.4-0.9 cm). See table 1 below.

Table 1. FNA Diagnoses in 2335 Thyroid Nodules

	Benign	Atypical/ Suspicious	Follicular Lesion/ Neoplasm	Papillary Carcinoma	Others*	Total
Nodule < 1.0 cm	140 (95.9%)	1 (0.7%)**	1 (0.7%)***	4 (2.7%)	0 (0%)	146
Nodule ≥ 1.0 cm	2051 (93.6%)	15 (0.7%)	67 (3.1%)	54 (2.5%)	2 (0.1%)	2189
Total	2191(93.8%)	16 (0.7%)	68 (2.9%)	58 (2.5%)	2 (0.09%)	2335

* Including 1 anaplastic and 1 poorly differentiated follicular ca, **Suspicious for papillary ca, ***Follicular neoplasm

Conclusions: 1. In this study, with the current management recommendations, the incidence of papillary carcinoma was found to be similar in micronodules and nodules measuring ≥1.0 cm. **2.** Follicular neoplasms were extremely rare in nodules less than 1.0 cm**. 3.** More aggressive forms of thyroid cancer were not detected at all in micronodules.

354 Comparison of Esophageal Brushing Cytology with Gastroesophageal Biopsy in Detecting Genomic Mutations Important in Malignant Transformation

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Background: Esophageal brushing cytology (EBC) and gastroesophageal biopsy (GEB) are complementary procedures for the evaluation of gastroesophageal lesions that help guide patient surveillance and treatment. In this study, we evaluated which of EBC or GEB is more effective in demonstrating genomic mutations important in malignant transformation.

Design: 34 GEBs with concomitant EBCs were divided into 5 groups: group 1: normal mucosa in EBC and GEB; group 2: Intestinal metaplasia (IM) in EBC and GEB; group 3: IM with mild atypia in EBC and IM with low grade dysplasia in GEB; group 4: IM with severe atypia in EBC and IM with high grade dysplasia in GEB; Group 5: adenocarcinoma in EBC and GEB. Representative cells were microdissected and DNA from these cells were extracted from both the EBC smears and GEB specimens. LOH was quantitatively determined for a broad panel of 17 microsatellite repeat markers near 10 tumor suppressor genes by PCR with labeled oligonucleotides followed by automated capillary electrophoresis.

Results:

Accumulation of genetic mutatins detected in EBC and GEB

Group	No.	Mutations in EBC	Mutations in GEB	P Value
Group 1	10	0.60 ± 0.52	0.00 ± 0.00	0.0051
Group 2	11	20.9 ± 1.64*	0.45 ± 0.69*	0.0026
Group 3	5	4.20 ± 0.84*	2.40 ± 0.89*	0.0008
Group 4	4	6.00 ± 0.82*	4.75 ± 1.50*	0.0796
Group 5	4	6.75 ± 1.26	5.25 ± 1.50	0.1027

*: $P < 0.01$ or < 0.05, as compared with the group above it.

Conclusions: 1. There is a progressive accumulation of genomic mutations from benign IM to adenocarcinoma in both EBC and GEB. 2. EBC appears to be more informative than GEB in demonstrating genomic mutations in IM and low grade dysplasia, but not in high grade dysplasia and adenocarcinoma. 3. LOHs at 9p21 (p16 and p14arf), 10q23 (pTEN), 17p13 (p53), 17q12 (HER2/neu, NF1) and 22q13 (NF2) begin to accumulate in IM, possibly reflecting their role in IM. 4. Frequency of LOHs at 1p36 (CMM1 and L-myc) and 17p13 (p53) increase significantly in dysplasia, possibly reflecting their role in dysplasia. 5. LOHs at 5q23 (APC, MCC) and 17q21 (NME1, BCRA1) increase significantly in adenocarcinoma, possibly reflecting their role in malignant transformation.

355 Immunodetection of p16/ProExC in Liquid-Based Cytology Specimens on Cell Block Sections – To Search for a Better Way on Screening of Pap Smears

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Background: Our previous study showed a cocktail of p16 (Dako)/ProExC (MCM2 and DNA topoisomerase IIA [TriPath]) to be a highly sensitive marker in confirming the diagnosis of both low-grade and high-grade squamous intraepithelial lesions (SIL) on cervical biopsy specimens (Human Pathology; 2007:1335-1344) and in a small number of liquid-based cytology specimens on cell block sections (CBS) (Modern Pathol 2007;20,#326). In this study, we evaluated the same cocktail in a larger series of similarly prepared specimens.

Design: Three hundred forty-eight (348) liquid-based cytology specimens were prepared for CBS. Five categories of cases were included in this study: Group #1 (G1) – 65 cases of high-grade squamous intraepithelial lesions (HSIL); Group #2 (G2) – 153 cases of low-grade squamous intraepithelial lesions (LSIL); Group #3 (G3) – 39 cases of atypical squamous cell cannot exclude a high grade lesion (ASC/H); Group #4 (G4) – 23 cases of atypical glandular cells (AGC); Group #5 (G5) – 68 negative/reactive cases. Immunostaining with monoclonal antibodies against the p16/ProEXC mixture was 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: The results are summarized in Table 1. Pap smear diagnosis served as a standard for comparison. Among 8 p16/ProExC negative cases in G1 (HSIL), two of them showed false negative results with low cellularity and the remaining 6 cases did not have followup cervical biopsy data. Most positively stained cells in G5 (negative/reactive) were metaplastic squamous cells, endocervical cells, and endometrial cells.

Table 1. Comparison of Pap Smear Diagnosis to Positive Immunostaining Results

Pap Smear Diagnosis	p16/ProExC -Positive	p16/ProExC -Negative
HSIL (N=65)	57 (87.7%)	8 (12.3%)
LSIL (N=153)	85 (55.6%)	68 (44.4%)
ASC/H (N=39)	26 (66.6%)	13 (33.3%)
AGC (N=23)	15 (65.2%)	8 (34.8%)
Negative (N=68)	12 (17.6%)	56 (82.4%)

Conclusions: Our data indicate that a cocktail of p16/ProExC provides high diagnostic sensitivity for cervical dysplasia and can potentially serve as a novel marker for screening Pap smears. Collection of followup biopsy data to confirm the diagnosis is currently undertaken.

356 High Grade Urothelial Carcinoma: Comparison of Cytospin with Surepath Liquid Based Processing

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Background: Urinary cytology has played an important role in the detection and follow-up of individuals with high-grade urothelial carcinoma (HGUC), including the clinically silent flat carcinoma in-situ. There have been many studies comparing the liquid based ThinPrep method with cytospins for urine processing, however, none for SurePath processing.

Design: 21 urinary tract cytology cases (11 bladder washings, 5 voided urine, 2 catheterized urine, 2 renal washings, 1 further unspecified renal pelvis collection) of HGUC were assessed on SurePath and cytospin slides. The cytologic characteristics like cellularity (20 malignant cells for adequacy), cell morphology and background were assessed.
Results: Cellularity: 11 cases had greater cellularity by SurePath than by cytospin. 10 cases showed similar cellularity on both SurePath and cytospin.**Cell Morphology**: 18 cases showed pleomrophism on both SurePath and cytospin. 2 cases pleomorphism was present on SurePath, but not on cytospin. 1 case, variability was minimal to absent on both SurePath and cytospin. **Cytoplasmic Features**: Denser cytoplasm was present in all 21 Surepath cases **Nuclear Features:** 20 cases showed large nuclei with irregular contours and high N:C ratio on both SurePath and cytospin. 1 remaining case did not meet these criteria on cytospin. 17 of 21 cases, nuclear hyperchromasia was more pronounced on SurePath. In the remaining 4 cases, nuclear hyperchromatism was more pronounced on cytospin. 17 of the 21 cases exhibited macronucleoli on both SurePath and cytospin. 3 other cases lacked macronucleoli by either processing technique. The 1 remaining case demonstrated macronucleoli on SurePath, but not on cytospin. **Mitotic Activity**: 7 (4 on Surepath and 3 on cytospin) of 21 cases had mitotic figures. **Background**: 13 of 21 cases had lesser inflammation, necrosis, and hemorrhage on SurePath than on cytospin. 4 other cases showed a greater degree of these background features on SurePath than on cytospin. 4 remaining cases had a similar degree of these background features by both processing techniques.
Conclusions: SurePath had greater cellularity and nuclear hyperchromasia for HGUC. SurePath had less background inflammation, necrosis, and/or hemorrhage, allowing easier detection of isolated malignant cells. Even though SurePath demonstrated a greater degree of nuclear hyperchromasia in most of the cases, the darker quality of the nuclei increased the difficulty of finding macronucleoli. Cytospins had crisper and more translucent nuclear features, making it easier to detect macronucleoli.

357 Increased Rate of Atypical Squamous Cells of Undetermined Significance and Declining High-Risk Human Papillomavirus Rates Following Implementation of ThinPrep Imaging System (Imager)

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Background: The ThinPrep Imaging System (Imager) for cervical cytology is being increasingly employed in many US laboratories, but the effect of the Imager stain on the rate of atypical squamous cells of undetermined significance (ASCUS) interpretations has not been independently reported. The Imager stain is a proprietary, pre-formulated stain that allows measurement of cellular DNA content. The purpose of this study is to evaluate the effect of implementing the Imager stain on ASCUS rates of ThinPrep Pap tests.
Design: A total of 265 Imager stained ThinPrep ASCUS cases from the first five months of Imager use were reviewed. All cases were analyzed for high-risk human papillomavirus (hr-HPV) using the Hybrid Capture II (HCII) assay (Beckton-Dickson). Being an objective test for the presence of hr-HPV, the rate of hr-HPV positive cases was used to monitor the quality of ASCUS interpretations. The hr-HPV positive rate was calculated overall for the 5-month period and for each of three pathologists reviewing cases in this time period. The hr-HPV positive rate was compared to ThinPrep ASCUS cases from the equivalent period of the previous year without the use of the Imager stain. All cases of ASCUS with negative hr-HPV test results were reviewed by two pathologists (AL and RKP) to evaluate potential factors involved in ASCUS misinterpretation.
Results: The proportion of ASCUS diagnoses increased after Imager stain implementation, from 1.79% to 3.14% (Chi-Square=41.07, $p < 0.001$). However, the hr-HPV positive rate for ASCUS cases decreased overall from 61.3% without Imager stain to 53.6% with Imager stain (Chi-Square=2.644, p=0.104). Review of hr-HPV-negative Imager stain ASCUS cases demonstrated that increased nuclear hyperchromasia, particularly in squamous metaplastic cells, was the most common reason for over-interpretation of a negative test as ASCUS, accounting for 23.5% of cases.
Conclusions: Implementation of the Imager stain required for the ThinPrep Imaging System led to a significant increase in ASCUS rates while the hr-HPV positive rate decreased. Our results strongly suggest that the Imager stain may lead to over-interpretation of ASCUS. As a result, ASCUS rates may initially rise following the conversion to Imager staining. With implementation of the Imager stain, cytopathologists need to recalibrate their criteria for the ASCUS diagnosis, and in particular, not overinterpret ASCUS in cases with increased nuclear hyperchromasia in squamous metaplastic cells.

358 Causes of Negative FNA's Results in Patients with Pancreatic Neoplasm

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Background: The Whipple procedure (pancreatico-duodenectomy) is associated with significant morbidity and adverse outcomes for patients with pancreatic cancer. With the advent of Endoscopic Ultrasound (EUS) for pancreatic lesions, more pancreatic fine-needle aspirations (FNAs) are being performed. These FNAs pose unique diagnostic challenges for the cytopathologist given the high incidence of coexisting benign contaminant epithelium that may mimic neoplastic pancreatic lesions and mucin from the gastrointestinal tract. The aim of this study was to determine the rate and causes of negative FNAs in patients with a positive surgical follow-up.
Design: Between 2003 and 2006, we identified a total of 240 pancreatic resection procedures at our institution. Based on cytohistology correlation, false negative FNA cases (12cases) were identified of which seven lesions were from the head/uncinate, four from the body and one from a Klatskin's tumor. The cytology slides were examined for the presence of contaminant epithelium (normal gastric and duodenal epithelium) presence of mucin, and atypical cells (singly, or in clusters). Data were analysed using Stata 10 as necessary (StataCorp, College Station, TX).
Results: The mean age of patients in the cohort was 59.5 yrs (range=30-79 yrs). Radiologically, the lesions ranged in size from 1.2-4.6 cm (Mean 2.96), seven having a prominent cystic component. In all except one case (which was deemed unsatisfactory), cytology was signed out as negative. All cases except one had low cellularity. In four cases, contaminant GI epithelium was present, two from IPMNs, and two from adenocarcinoma with mucin production. The final histopathologic diagnosis was a mucinous tumor in six cases (including 3 IPMN and 3 Mucinous Cystic Neoplasms). The other six comprised five adenocarcinomas and one serous microcystic adenoma.
Conclusions: The false negative rate in our study was 12/240 (5%). With the increased use of EUS-guided aspiration cytology of pancreatic lesions, there is a need for better establishment of adequacy criteria in these FNAs: Low cellularity, presence of contaminant epithelium and abundant mucin (stemming from a cystic component due to IPMN) may all lead to false negative cytology. Better concordance and lowering of false negative cytology rate can be achieved by addressing these issues. The main cause of false negative results is an insufficient sampling of a lesion.

359 Importance of Digital Image Cytometry as an Adjunct to Cytological Diagnosis of Bone and Soft Tissue Tumors

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Background: Accurate classification of bone and soft tissue tumors (STTs) by fine needle aspiration (FNA) may require use of immunochemistry, FISH and cytogenetics in conjunction with morphology. Digital Image Cytometry (DIC) can provide additional information regarding biologic behavior based on parameters of Ploidy, Proliferation index (PI) and Percentage of cells with DNA exceeding 5c (>5c). We wished to study if DIC would contribute to the diagnosis and prognosis of Bone and soft tissue tumors.
Design: A SAMBA 4000 image analyzer was used to determine DNA content of Feulgen stained, interactively selected cells of cytologic preparations from 70 cases of STTs (mean age: 48 years; range: 3-84; 32 Females; 38 males). Clinical details including type of tumor, status of disease (primary, recurrent, metastatic), and survival for up to 15 years post surgery were obtained. Pearson's correlations and Levenes T-test was used to analyze the results.
Results: Statistical analysis showed that ploidy, PI, and % of cells>5C correlated with the histological grade of primary tumor ($p<0.000$). Liposarcomas tended to be diploid with low PI while MFHs were aneuploid with high PI and high percentage cells >5c. Liposarcomas were diploid ($p<0.01$) had a lower PI ($p<0.015$) and fewer cells with >5c compared to leiomyosarcomas ($p<0.000$), malignant fibrous histiocytoma (MFH) ($p<0.033$), and osteosarcoma ($p<0.002$). Rhabdomyosarcoma showed fewer cells with >5c than leiomyosarcoma ($p<0.032$). Mortality correlated with metastatic disease ($p<0.01$) but could not be predicted based on DIC results. There was no statistical difference in DIC results in comparing primary v/s metastatic disease, however, in some cases high PI was associated with local recurrence.
Conclusions: DIC demonstrated distinctive histograms characteristic for different types of STTs (see table). DIC is a good adjunct to cytology in the characterization of soft tissue tumors, but cannot be used to predict metastasis and survival.

Mean PI and percentage of cells with DNA exceeding 5c in different subtypes of Bone and Soft tissue tumors

Tumor type	Number of caes	Mean Proliferation index	Mean DNA>5c
Liposarcoma	15	5%	7%
MFH	15	8.4%	15.2%
Osteosarcoma	7	9.6%	27%
Leiomyosarcoma	5	8.9%	30.1%
Rhabdomyosarcoma	4	8%	8.9%
Angiosarcoma	3	9.4%	12.2%
other	49	N/A	N/A
Total	70		

360 Fluorescence In-Situ Hybridization (FISH) Studies on Direct Smears: An Approach To Enhance the Fine Needle Aspiration Biopsy Diagnosis of B-Cell Non-Hodgkin Lymphomas

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Background: Fluorescence in-situ hybridization (FISH), like flow cytometry, can be helpful in establishing B-cell clonality and subtyping B-cell lymphomas. In the fine needle aspiration biopsy (FNAB) diagnosis of lymphoma, the role of flow cytometry can be limited due to low cellularity, unusual immunophenotypic features, or absent surface immunoglobulin. Thus, the aim of this study is to determine if FISH studies performed on unstained direct aspirate smears can be a useful ancillary technique in the diagnosis of B-cell Non-Hodgkin Lymphomas (B-NHL).
Design: Between January 2005 and July 2007, 181 cases of B-NHL were diagnosed by FNAB at the University of Pittsburgh Medical Center-Shadyside Hospital. FISH studies were performed in 105 cases (58%) to assess the IgH gene rearrangement and/or other specific chromosomal translocation(s). We retrospectively reviewed these cases to see if the outcome of the FISH studies had additional diagnostic value to the flow cytometry and cytomorphology. The FISH results were classified as positive, negative, or indeterminate. Indeterminate cases consisted of cases that did not have a characteristic translocation, but did have a chromosomal abnormality of low level or unknown significance.
Results: The 105 cases with FISH studies included follicular lymphoma (36; 34.3%), diffuse large B cell lymphoma (30; 28.6%), B cell lymphoma NOS (22; 21.0%), mantle cell lymphoma (9; 8.6%), Burkitt lymphoma (5; 4.8%) and small lymphocytic lymphoma (3; 2.9%). The most common indication for performing FISH studies was for subclassification (63 cases; 60%), followed by negative/insufficient flow cytometry results (39 cases, 37%). Of the 105 cases, FISH was positive in 65 (61.9%) cases, negative in 30 (28.6%) cases and indeterminate in 10 (9.5%) cases. Of the 65 positive FISH cases, 25 (38.5%) cases were negative by flow cytometry due to insufficient cells

for analysis (14; 21.5%) or the inability to establish clonality in surface immunoglobulin negative cases (11; 16.9%). Of the 63 cases submitted for further classification, 23 cases (36.5%) were successful in subclassifying the lymphoma.
Conclusions: This study illustrates that FISH studies performed on direct smears play a complementary role to the flow cytometry studies in establishing FNAB diagnosis and/or subclassification of B-NHL. Thus, it is important to prepare adequate unstained smears at the time of the FNAB, which may aid in rendering an accurate diagnosis of B-NHL.

361 AGC in Cervical Cytology: A Comparison between Conventional Smear, ThinPrep, and SurePath

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Background: Pap smears are primarily a screening test for Squamous Intraepithelial Lesions (SIL) of the uterine cervix. However, the incidence of primary and metastatic adenocarcinoma of the cervix is increasing. Liquid Based Preparations (LBP) have been shown to be more predictive of glandular malignancy than Conventional Smears (CS). To the best of our knowledge, no single comprehensive long-term study compared the prevalence and predictive value (PV) of Atypical Glandular Cells (AGC) between CS, ThinPrep (TP), and SurePath (SP).
Design: Our institutions Cytopathology records of 1,352,061 Pap smears done between November 1999 and September 2007 including 1,186,665 TP, 147,224 CS, and 18,172 SP were searched for all results of Atypical Glandular Cells of Undetermined Significance (AGUS) and AGC, yielding 1208 cases (0.089%). Follow up tissue diagnosis was available for review in 700 cases. We compared the prevalence of AGC between TP, SP, and CS using a Chi-square test. In addition, for each type of preparation, we calculated the predictive value (PV) of a diagnosis of AGC for endocervical or metastatic adenocarcinoma using tissue diagnosis as a golden standard. We compared the PV of the 3 preparations using a Chi-square test. To avoid confusion, we replaced results originally reported as AGUS before 2001 by AGC (2001 Bethesda criteria).
Results: The Prevalence of AGC was 0.072% for TP, 0.22% for CS, and 0.077% for SP. AGC was significantly more prevalent in CS than both LBP types ($P<0.05$). The prevalence of AGC was higher in SP than in TP. This difference, however, was not statistically significant. The PV of AGC for adenocarcinoma was 16.36% in TP, 4.8% in CS, and 21.4% in SP. Hence, AGC was significantly more predictive of adenocarcinoma in each LBP compared to CS ($P<0.05$). AGC was more predictive of adenocarcinoma in SP than in TP. This difference, however, was not statistically significant. The PV of AGC for SIL showed no significant difference between TP, CS, and SP (6.85%, 8.76, and 7.15, respectively).
Conclusions: In this study, the overall prevalence of AGC (0.089%) was lower than reported in the literature. The finding of AGC was significantly less prevalent in both LBP types than in CS. There was no such significant difference between ThinPrep and SurePath. In addition, the finding of AGC in LBP was significantly more predictive of cervical or metastatic adenocarcinoma than in CS. No such difference was observed between ThinPrep and SurePath. No preparation was superior to another in distinguishing SIL from glandular lesions.

362 False Negative Sentinel Lymph Nodes by Intraoperative Cytologic Evaluation: A Single Institutional Study of 1122 Breast Cancer Patients

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Background: The clinical significance of false negative (FN) intraoperative cytologic evaluation of sentinel lymph nodes (SLN) in breast cancer patients is not known. The aim of this study was to analyze the clinicopathologic characteristics of these cases with particular emphasis on 'missed' macrometastases.
Design: Of the 1144 SLN cases from 1122 breast cancer patients evaluated intraoperatively by cytology from 2003 to 2007, there were 286 (25%) histologically confirmed positive SLN cases. The study cohort was comprised of 85 cases originally interpreted as negative on cytology but proven positive on histology (FN). The FN macrometastasis subgroup (N=19) was compared with the true positive (TP) macrometastasis subgroup (N=160) in terms of size of SLN, number of positive SLN, size of macrometastasis in the SLN, status of completion axillary lymph node dissection (ALND) and size of primary breast tumor on resection. The original Papanicolaou stained smears prepared by scraping the bisected SLN, 3 H & E stained histologic levels and immunohistochemical stain AE1/3 for negative cases, were reviewed. The pathology reports were reviewed for size of breast tumor and ALND status.
Results: Of the 85 FN cases, there were 19 (22%) macrometastases, 34 (40%) micrometastases and 32 (38%) cases with isolated tumor cells (ITC) (20 cases by immunohistochemistry only and 12 by H & E). Completion ALND was performed in 17 of the 19 FN macrometastasis cases. Although 4 of the 17 patients had positive ALND, only 1 case had a macrometastasis in the ALND. The FN macrometastasis subgroup was similar to the TP macrometastasis subgroup in mean size and number of positive SLN and total number of SLN submitted for cytology. However, the FN macrometastasis subgroup had smaller primary breast tumors (mean 19 mm vs 24 mm), smaller size of SLN macrometastases (mean 4.8 mm vs 9.8 mm) and lower rate of positive ALND (24% vs 56%).
Conclusions: Although the overall FN rate of intraoperative cytologic evaluation of SLN in our institution was 23%, the FN rate for missed macrometastases was only 6%. The FN macrometastasis group tended to have smaller primary tumors, smaller SLN macrometastases and negative completion ALND compared to the TP macrometastasis group. The FN rate of intraoperative SLN evaluation by cytology is dependent on various factors including size of SLN metastasis and extent of histologic evaluation of SLN including performance of immunohistochemistry on H & E negative cases.

363 Development of a Virtual Web-Based External Quality Assessment [EQA] Scheme in Cytopathology

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Background: External quality assessment [EQA] is recognised as central to a quality management system in cytopathology. The current system of EQA employed by most laboratories includes circulation of slides containing biological material, which are prone to breakage and loss, constituting a significant risk in relation to governance of the patient hospital record. To overcome these difficulties, CERVIVA have developed a novel scanning and virtual web-based EQA platform for cytopathology. Digital Slide Box (DSB) is an online digital slide management system, built upon the latest Web 2.0 technologies (PHP, JavaScript, XML, Flash). User data is stored within a back-end MySQL database, while images are stored on an ancillary warehouse. Image tiles are retrieved into DSB by Digital Slide Server (DSS), the image-handling component of DSB, minimizing bandwidth required for distribution over the web.
Design: Three centres participating in a pilot study in Dublin have commenced a slide circulation scheme. In each centre, 3 cytologists review ten peer-selected thin-prep specimens using digital slides. Each specimen was digitised in 5 focal planes across a total depth of 10µm, with a Hamamatsu Nanozoomer Digital Pathology System using a 40x / 0.75 NA objective lens. Cytologists review the digital slides using SlidePath's DSB workstations.
Results: Results of the initial technical evaluation indicate that this platform is suitable for use in EQA formats with cytopathology material, obviating the need for slide circulation between laboratories. Preliminary results suggest that the EQA formats may prove to be a valuable audit and educational tool in cytopathology laboratories.
Conclusions: The DSB EQA platform offers for the first time a comprehensive solution for EQA in cytopathology. The platform offers an integrative tool to assess performance, competence and proficiency in relation to slide analysis. Its capacity to deliver multi-focal plane slides for cytopathology EQA over the web is a key enabler of this new approach. In the future, it is anticipated that the platform will allow integration of decision making analysis in real-time. CERVIVA is supported by The Health Research Board, Ireland.

364 Should Ultrasound Guided Fine Needle Aspiration of Thyroid Replace Superficial Aspiration? Evaluation of a Decade of Practice

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Background: Fine needle aspiration (FNA) is the most accurate and cost effective method in the evaluation of thyroid nodules. Traditionally superficial FNA was used for palpable nodules (>1 cm) and ultrasound-guided FNA (USG) for non-palpable nodules (<1 cm). This study evaluates the change in the practice of thyroid FNA following the introduction of USG in an academic institution with an active pathology-driven FNA service.
Design: Thyroid FNAs performed at our institution in 1995 and 2005 were retrieved (we allowed 18 months for possible surgical follow-up in the most recent cases). The method of sampling was recorded as "superficial" if performed by pathologist and "ultrasound" if performed using USG. Diagnoses were classified: unsatisfactory (no colloid/ follicular cells); non-neoplastic thyroid (NNT) (goiter, adenomatous nodule, colloid nodule, thyroiditis); cellular follicular lesion, favor neoplasm (CFL); and suspicious/positive for carcinoma. Cases with descriptive interpretations were included as NNT. The referring clinician's speciality and surgical follow-up were recorded.
Results: Within an interval of 10 years the ratio of superficial vs. USG thyroid FNA has markedly changed. In 1995 > 90% of FNAs were performed by pathologists. In 2005 more than half (52.8%) of cases were USG. The distribution of diagnoses has not changed (see table). 5.2% of superficial FNAs in 2005 were unsatisfactory, however 3/4 were benign on repeat FNA.

	1995 (114 cases)		**2005 (161 cases)**	
Diagnosis	103 superficial (90.3%)	11 US (9.7%)	76 superficial (47.2%)	85 US (52.8%)
Unsatisfactory	0 (0%)	0 (0%)	4 (5.2%)	0 (0%)
NNT	91 (88.4%)	10 (90%)	65 (85.5%)	80 (94.2%)
CFL	4 (3.8%)	0 (0%)	5 (6.5%)	3 (3.5%)
Suspicious/positive	8 (7.8%)	1 (10%)	2 (2.8%)	2 (2.3%)
Surgical Follow up	25 (24%)	2 (18%)	19 (25%)	10 (11.7%)
Referring Clinician				
Endocrinologists	47 (45.6%)	9 (81.8%)	33 (43.4%)	37 (43.5%)
ENT/general surgeon	25 (24.3%)	0 (0%)	21 (27.7%)	17 (20%)
Other	31 (30.1%)	2 (18.2%)	22 (28.9%)	31 (36.5%)

Conclusions: Within 10 years thyroid FNA has shifted from superficial to USG and has been embraced equally by endocrinologists and clinicians in other specialties. However, there has been no change in the distribution of diagnoses or percentage of cases that underwent surgical excision. Our study suggests that the usage of USG FNA for all thyroid nodules (both palpable and non-palpable) does not produce superior results compared to superficial FNA.

365 Papillary Thyroid Carcinoma Arising within Follicular Adenoma: A Masked Cytomorphologic Analysis of 17 Cases

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Background: Papillary thyroid carcinoma arising in a follicular adenoma (PTCFA) is a well recognized, but rare histopathologic entity. However, the cytologic features of this subset of papillary thyroid carcinomas (PTC) have not been previously described.

We propose that this uncommon presentation of PTC is difficult to recognize by fine needle aspiration biopsy (FNAB) and contributes to a subset of thyroid aspirates interpreted as "atypical".

Design: 17 FNAB cases diagnosed as "papillary thyroid carcinoma arising in a follicular adenoma" on corresponding surgical excision were identified from the archival records of 2 large urban hospitals. A control group of 40 FNABs comprised of 20 follicular adenomas (FA) and 20 PTCs were identified (based on the corresponding surgical pathology diagnosis) for comparison. All 57 FNABs were reviewed in a masked fashion, and scored for a series of 31 cytomorphologic features.

Results: Aspirates of PTCFA were originally interpreted as "negative" (n=3), "atypical/ suspicious for a follicular neoplasm" (n=6), "atypical, cannot exclude PTC" (n=4), "suspicious for PTC" (n=3), and "positive for PTC" (n=1). On masked review, the most common cytomorphologic features of PTCFA were a solid or mixed cytoarchitectural pattern (65%), nuclear grooves (94%), a spectrum of round to oval nuclei (41%), micronucleoli (76%), and absence of intranuclear pseudoinclusions (88%). In most cases, the chromatin pattern varied from coarse and dark to pale. Relative to the 20 FA controls, the 10 PTCFA cases diagnosed as "atypical" more frequently exhibited oval nuclei, pale chromatin, nuclear grooves, and micronucleoli.

Conclusions: PTCFAs represent a rare subset of PTCs that are difficult to recognize by FNAB. Most cases exhibit overlapping features between a follicular neoplasm and conventional PTC. They are often interpreted as "atypical", but differ from FAs by the presence of one or more of the following: oval nuclei, pale chromatin, nuclear grooves, and micronucleoli.

366 Extrauterine Adenocarcinomas Detected on Cervical Pap Smears: Cytomorphologic Features in Conventional and Liquid-Based Smears

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Background: Extrauterine adenocarcinomas (ACs) are rarely detected on Pap (Papanicolaou) smears. The Bethesda System reported the diagnostic criteria, however, their distinction from uterine adenocarcinomas may be problematic. Most published reports are only on conventional smears (CS) and in the form of single case reports. Their cytologic features on liquid-based smear (LS) in comparison with CS have not been studied.

Design: Twenty seven cervical Pap smears with malignant cells originating in histologically proven extrauterine ACs were identified at gynecologic oncology referral centers. ACs with direct invasion from adjacent organs were excluded. Twelve cases were CS, and 15 LS. All smears were reviewed and evaluated for 1) cellularity, 2) background, 3) architecture, 4) nuclear size. Clinicopathologic information of the primary tumors was obtained.

Results: The patient's age ranged from 22 to 72. Eighteen (67%) smears were sampled as a work-up before the surgery, five were investigation for tumors with unknown origin, 4 were as follow-up for the original tumors. The primary sites included ovary, primary peritoneal carcinoma, breast, colon, and unknown primary. Cervical mucosa or endometrium was involved in 7 cases (26%), fallopian tube in 7 cases (26%), and peritoneal disease was seen in 24 cases (89%). The original cytologic diagnoses were ACs (17), metastatic ACs (8), endometrial AC (1), endocervical AC (1).

Cytomorphologic comparison between CS and LS

	CS			LS		
	Ovary (8)	Primary peritoneal (2)	Unknown primary (2)	Ovary (12)	Breast (2)	Colon (1)
High cellularity	5	1	1	5	0	1
Clean background	6	2	1	9	1	0
Most common architecture	CB	CB	Large cluster, CB	CB, Papillary	Loose cluster	Syncytial
Nuclear size	1-3x	1-2X	1-1.5X	1.5-3X	2-3X	2-3x

CB: cell ball-like clusters, *>20 clusters per slides, ** relative to intermediate squamous cells

Conclusions: 1) The presence of malignant cells is mostly associated with peritoneal disease, and the ovary is the most common primary site 2) Overall cytologic features are similar on between CS and LS, and three-dimensional CB with clean background is predominant pattern. Cellularity can be high and is not associated with the extent of disease. 3) Adenocarcinoma is easily identifiable, but primary site cannot be definitely specified on cytology alone in the majority cases.

367 Routine Assessment of Peripheral Lung Lesions with Touch Preparations of CT-Guided Needle Core Biopsies (NCB) Instead of Fine Needle Aspiration (FNA) Avoids Ambiguous Results

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Background: Pathologic diagnosis of peripheral lung lesions is often made with CT-guided FNA. NCB, widely considered more definitive, is sometimes performed concurrently with the FNA, but such concurrent testing can lead to discordant or ambiguous results on the same specimen, increased costs, duplication of work, and increased risk of procedural complications such as pneumothorax due to repeated pleural puncture. In lung lesions associated with multiple malignancies, where immunohistochemistry (IHC) is required to identify the primary site, a conclusive diagnosis is sometimes impossible with traditional FNA, due to scant material on the smears and the cell block. Therefore, in our institution we instituted a protocol for diagnosis of lung lesions that consisted solely of CT-guided NCB that were assessed for adequacy using touch-preparations (NCB-TP).

Design: CT-guided NCB of peripheral lung lesions were performed using 20 g needles. On-site assessment of air dried, diff quick stained TP was performed by a Cytopathologist who assessed specimen adequacy and determined the need for microbiological culture and flow-cytometric analysis. NCB were processed for H&E stains. IHC was requested when needed. We performed a computerized search of our laboratory information system to identify all CT-guided lung FNA/ NCB-TP over two years. Data was entered into an Excel spreadsheet for analysis.

Results: Over two years, 125 cases of CT-guided NCB-TP were performed (Table 1). Twenty-eight patients had a h/o multiple malignancies. Eight cases were non diagnostic (fibrosis/necrosis). Of the remaining 117 cases, ambiguous diagnosis were minimal with two cases diagnosed as suspicious for malignancy (1.7%). Seventy-seven % were positive for malignancy with primary lung adenocarcinoma being the commonest malignancy (42.9%). 7.6% were metastatic carcinomas. IHC was performed on 77 cases.

Number of cases in different categories

Non diagnostic (necrosis/ fibrosis)	8
Negative for malignancy	5
Infection/ inflammation	13
Carcinoid tumor	1
Atypical proliferation, suspicious for bronchiolo-alveolar carcinoma	5
Suspicious for malignancy	2
Positive for malignancy	91
n=	125

Conclusions: With improving radiologic methods and availability of thin needles, adequate samples from lung lesions can routinely be obtained with NCB-TP, with elimination of ambiguous (e.g. atypical/ suspicious) cytology diagnoses, reduced cost and time, and lower risk to the patient.

368 Diffuse Strong p16INK4A Staining Distinguishes Cystic Oropharyngeal Squamous Cell Carcinomas from Branchial Cleft Cysts

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Background: Squamous cell carcinoma (SCC) of the head and neck commonly presents as cystic masses in the neck. Distinction of cystic SCC from benign squamous cell lesions, such as branchial cleft cysts (BCC), can be very challenging on fine needle aspiration biopsy specimens (FNAB). The aims of this study are to investigate the utility of p16INK4A staining in distinguishing benign from malignant cystic squamous lesions and to further characterize the cytomorphologic features of HPV-related SCC.

Design: Twenty-six cases of cystic metastatic SCC comprise this study, including 15 excisions and 11 FNAB: oropharnyx (11 non-keratinizing (NK-SCC), 4 keratinizing (K-SCC)), oral cavity (4 K-SCC), larynx (4 K-SCC), and unknown (1 NK-SCC, 2 K-SCC). Twenty-five BCC cases (20 excisions and 5 FNAB) were also studied. All cases were evaluated for p16INK4A expression (Dako, Carpinteria, CA) with staining scored as strong, weak, or negative and as either diffuse (greater than 80% cells) or focal (5-80%). Forty-one of the 51 total cases had sufficient material to evaluate for HPV viral DNA by PCR and pyrosequencing.

Results: A summary of the p16INK4A immunohistochemical staining and HPV genotyping is listed in the table below.

p16INK4A and HPV Genotyping of Cystic SCC and BCC

	Oropharynx NK-SCC	Oropharynx K-SCC	Oral Cavity K-SCC	Larynx K-SCC	Unknown SCC	BCC
Diffuse Strong p16INK4A	11/11	1/4	0/4	0/4	2/3	0/25*
HPV Genotype	8/9 HPV16	0/2	1/4 HPV16/18	0/3	2/3 HPV16	7/20 (4 HPV18, 3 HPV16/18)

*6/25 BCC cases exhibited focal, strong p16INK4A staining involving the superficial squamous cells lining the cyst.

Conclusions: Oropharyngeal primary SCCs commonly give rise to cystic metastases in the neck exhibiting a non-keratinizing cytomorphology. The presence of diffuse, strong p16INK4A supports the diagnosis of SCC and suggests an oropharyngeal primary. However, the detection of HPV 16 or HPV 18 alone is not specific for a diagnosis of malignancy as a significant number of BCC cases were found to be infected with HPV16 and/or HPV18. The pattern of p16INK4A reactivity with cases of BCC was different and characterized by focal, strong p16INK4A staining involving the superficial squamous epithelium and not the basal layer. This distinction can be helpful in the evaluation of cytomorphologically equivocal squamous lesions.

369 Association between Age and HIV Status among Women with High Risk HPV+ ASC-US Pap Tests

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Background: High-risk human papillomavirus infection (HR-HPV) is a precursor to cervical cancer, and its predictive value is higher in women over 30 years old. HIV infection is associated with an increased risk of cervical cancer and precancer.

Design: Retrospective review of the HIV status of ASC-US HR-HPV+ patients by age <40 or ≥40 in our inner city hospital was performed. The Digene Hybrid Capture II assay was used to determine HR-HPV infection.

Results: HIV positivity was seen in 67/319 (21%) of the population diagnosed with HR-HPV. The HIV status by age category is presented in Table 1.

Table 1. HIV status by age groups in HR-HPV positive patients

	HIV +	HIV -	TOTAL
<40	27(11%)	218(89%)	245
≥40	40(54%)	34(46%)	74
TOTAL	67(21%)	252(79%)	319

$p < 0.001$(Chi-square, DF = 1)

Conclusions: Women 40 years and older with ASC-US Pap tests who are also HR-HPV+ are significantly more likely to be HIV+ than those under 40. This retrospective study is limited by selection bias; prospective studies of the HIV status of older HR-HPV+ patients are needed to determine if a different protocol of HIV screening is warranted for this sub-population.

370 Does the *ThinPrep Imaging System* Increase the Detection of High Risk HPV Positive ASC and AGUS? The Women & Infants Experience with over 200,000 Cervical Cytology Cases

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Background: Published reports have demonstrated that introduction of the ThinPrep Imaging System (Imager) to the cytology screening services has increased the detection rate of high grade squamous intraepithelial lesions (HSILs). In accordance with recent clinical treatment guidelines, patients with atypical squamous or glandular cells of undetermined significance (ASC or AGUS) are often tested for high risk HPV infection using the Hybrid Capture HPV DNA test. Our high volume cytology laboratory, which serves a relatively stable population of patients with excellent clinical followup, introduced the Imager approximately two years ago. Our pathology and cytotechnology staff has undergone no major change in personnel, providing relatively stable continuity in diagnostic criteria, and our clinicians follow standard guidelines for reflex HPV testing. Therefore, we took the opportunity to investigate whether the Imager had resulted in any significant differences in our diagnostic categories, as well as whether the Imager increased the detection of high risk HPV DNA positive (HRHPV+) ASC or AGUS.

Design: Cytology cases with the diagnosis of ASC (ASC-US and ASC-H) and AGC were retrieved from the archival files of our institution during periods of 11 months prior to and 11 months after introduction of the Imager. All cytology materials were examined by the same staff of cytopathologists and cytotechnologists during the study period. The total number of cases in each category was correlated with results of reflex high risk HPV DNA testing when the latter were available. Statistical analyses were performed using the Chi-Square test.

Results:

Table

		Pre-Imager	Post-Imager
Total Cases		108,371	104,555
ASC		5884 (5.4%)*	5559 (5.3%)*
	Reflex HPVDNA Testing	5,536	5,515
	HPV+	38%*	34%*
AGUS		158 (0.14%)*	126 (0.12%)*
	Reflex HPVDNA Testing	116	102
	HPV+	14%**	23%**
HSIL		249 (0.23%)*	260 (0.25%)*
ASC/SIL Ratio		1.9	1.6

*p>0.05; **p <0.032

Conclusions: The ASC and AGUS rates did not change statistically before and after introduction of the Imager in our cytology laboratory. Although use of the Imager did not increase detection of HPV+ ASC, it does appear to increase significantly the detection rate of HPV+ AGUS.

371 Fine Needle Aspiration Cytology of Kidney Masses: A Retrospective Analysis of 304 Cases

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Background: The widespread use of transabdominal ultrasound (U/S) and computed tomography (CT) has led to an increased identification of renal masses. Fine needle aspiration (FNA) cytology performed under imaging guidance is a highly accurate method of establishing a preoperative diagnosis of malignancy in such patients.

Design: A computerized search of our laboratory information system was performed for a 16-year period from September 15, 1992 through September 15, 2007 and all kidney FNA cases were identified. Only FNA cases of solid renal masses or partially solid/cystic renal lesions were included in the study. FNA cases of purely cystic lesions of the kidney were excluded. The cytology reports from these cases and all available correlating surgical pathology reports were reviewed. A retrospective microscopic analysis of both cytology and correlating surgical pathology cases was performed in selected cases.

Results: Of the 304 FNA cases, there were 121 cases with histologic follow-up (40%). The cytologic diagnoses for the 183 cases without correlating surgical pathology were as follows: malignant: 113, suspicious for malignancy: 17, atypical cells identified: 8, specific benign lesions: 11, negative for malignancy: 26 and unsatisfactory: 8. The cytologic diagnoses for the 121 cases with histologic follow-up were as follows: malignant: 81, suspicious for malignancy: 15, atypical cells identified: 4, specific benign entity: 3, negative for malignancy: 13, and unsatisfactory: 5. Histologic follow-up demonstrated malignancy in 80 of 81 cases diagnosed by FNA as malignant, 15 of 19 cases diagnosed as suspicious or atypical, and in 10 of 21 cases diagnosed as benign, negative or unsatisfactory. The one false-positive FNA that was diagnosed as carcinoma proved to be an oncocytoma with cytologic atypia.

Conclusions: False-positive kidney FNA diagnoses were exceptionally rare with only 1 case occurring during the past 16 years. Most cases designated as suspicious for malignancy or containing atypical cells proved to be malignant and benign diagnoses did not exclude the possibility of malignancy. While adherence to strict cytologic criteria reduces false-positive diagnoses, this approach may have contributed to the underdiagnosis of malignancy in some cases. In summary, a kidney FNA diagnosis of malignancy is highly reliable and can be used to guide patient management, while all other cytologic diagnoses must be interpreted within the context of the patient's clinical presentation.

372 Significance of the Atypical Category in Urine Cytology: An Interobserver Study

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Background: A recent metaanalysis indicates that the sensitivity of voided urine cytology for grade 3 transitional cell carcinoma (TCC) is 60%. At this institution the sensitivity of a single voided urine cytology for grade 3 TCC is 69%, while 23% of these samples are deemed atypical. We performed an interobserver reproducibility study to evaluate a cohort of atypical urine samples from individuals with grade 3 TCCs.

Design: 11 consecutive voided urine cytology samples classified as atypical, on whom a subsequent bladder biopsy documented a grade 3 TCC, were retrieved from our files. The control group was composed of 9 voided urine cytology samples, all of whom had a prior history of grade 3 TCC, and were biopsy and cystoscopically negative for TCC on a follow-up of greater than 1 year. The analysis was conducted on digital whole slide images of ThinPrep slides (Aperio Scanscope CS system, Aperio Technologies, Inc.). Potential neoplastic cells were evaluated and annotated and observers were asked to classify all samples using the standard 4 grade classification system. For this analysis, negative and atypical samples were grouped together (group A), as were suspicious and positive cases (group B). The observers also documented the specific images and cytologic criteria on which their diagnosis of malignancy was based. Cell sizes were measured in selected cases.

Results: The number of annotated images varied from 8 to 146 (median 87). The interobserver reproducibility in our study varied from poor to moderate (kappa- 0.2 to 0.5) with a median kappa of 0.18 (slight agreement). The sensitivity varied from 28% to 91% (median 55%). The specificity varied from 33% to 100% (median 67%). In the majority of group B cases, the malignant diagnosis was based on single cells with high nuclear to cytoplasmic ratios and hyperchromasia. The cell size in cases with the highest degree of interobserver concordance for malignancy, was significantly greater (range 14µm -61µm; average 26µm) in comparison to the cell size from cases with the least degree of concordance for malignancy (range- 12µm to 22µm; average 13µm).

Conclusions: A significant minority of voided urine samples from biopsy proven grade 3 TCCs could not reproducibly be identified on cytology. The high levels of discordance justifies the continued use of the atypical category.

373 Comparison of Analytical Sensitivity and Specificity for HPV Detection by Chemiluminescent Nucleic Acid Testing and PCR-Based Methods

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Background: It is now well accepted that the high-risk Human Papilloma Virus (HPV) is the major etiologic agent in the development of cervical cancer. In this study we compared the presence of high-risk HPV genotypes in cytology preparation by Hybrid Capture II assay (HC II) (Digene Corporation) and AMPLICOR HPV test (Roche Molecular Diagnostics, CA, USA).

Design: 81 cytology cases were collected and processed by Liquid-based cytology (Tripath Imaging System, NC, USA). The patients' age ranged between 18 and 86 years (mean 37.8, SD: 14.13). 24 (29.6%) cases had the diagnosis of NILM, 20 (24.7%) ASCUS, 23 (28.4%) LGSIL, 9 (11.1%) HGSIL, 1(1.2 %) ASC-H and 4 (4.9%) LGHG (at least LGSIL with few cells suspicious for HGSIL). The samples were analyzed for high- risk HPV by both Hybrid Capture II assay and AMPLICOR HPV test. All cases, except 29 (35%), had follow-up by cytology and/or biopsy within 0 to10 months of original pap smear.

Results: Of a total of 81 Liquid-based cytology samples, all had either a positive or negative results by AMPLICOR HPV test. There was only one case detected as borderline by HC II assay with negative cytology follow up in six months, which was excluded from the analysis. The overall sensitivity and specificity of AMPLICOR HPV test was 55% (95% CI: 28% -79%) and 85% (95% CI: 85%- 93%) and of HC II assay was 60% (95% CI: 31% - 81%) and 82% (95% CI: 69% - 91%), respectively. The total agreement between follow up diagnoses and AMPLICOR HPV test was 78.8% (95% CI: 65.9% - 87.7%) with a positive likelihood ratio of 3.727 (95% CI: 1.493 - 9.308) and negative likelihood of 0.532 (95% CI: 0.275 - 1.03). The total agreement between the follow up diagnoses and HC II assay was 78.4 % (95% CI: 65.3% - 871.5%) with positive likelihood ratio of 3.514 (95% CI: 1.512 - 8.167) and negative likelihood of 0.482 (95% CI: 0.223 - 1.044).

Conclusions: Both HC II assay and the AMPLICOR HPV test demonstrate comparable results and the difference between the detection of high-risk HPV is marginal. The clinical application of these two commercially available tests can be determined by financial availability and the reimbursement of the practicing organization

374 Anal Cytology in HIV Patients and Bethesda's Criteria: A VAMC Experience

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Background: HIV patients are at an increased risk of developing AIN and anal cancer. The purpose of this study is to report our experience with using the Bethesda diagnostic criterion in anal cytology, with attention to ASCUS and negative cases with follow up.

Design: 1) 263 anal pap smears of 142 HIV positive men were reviewed independently by two investigators using Bethesda's criteria. High risk HPV DNA test utilizing PCR was performed by ARUP laboratories. 2) 57 patients with an initially negative cytology with a follow up pap were reviewed. Two follow up groups were compared; those whose cytology remained negative on follow up screening (non converters) and those showing ASCUS or higher (converters) on subsequent samples. We examined HPV DNA, HIV viral loads (VL) and serum CD4 (within 4 months of the pap). ANOVA and a t-test were used for statistics.

Results: 1) 24 cases had ASCUS (9.2%) and 63 cases (24%) had SIL. The ASC/SIL+ ratio for the 5th and 95th percentile rates are 0.4 and 3.1 for liquid based preps and our experience shows a rate of 0.38(<5th percentile). 15 of ASCUS patients (62.5%) had high risk HPV DNA on PCR. The 2003 CAP comparison of cervical cytology and ALTS recently reported rate of HPV-positive ASCUS of 41-60% while our population shows a rate of 63%. ASCUS patients had a 50% progression rate to SIL within 1-2 years. 2) Among 57/142 patients who had an initial negative cytology 22 (38.6%) had a subsequent atypical finding [ASCUS(9), LSIL(8) or HSIL(5)] and 18 had high risk HPV on follow up. 35 patients (61.4%) remained negative in subsequent samples. VL was significantly higher in converters (mean 10560 copies/ml) than non converters (mean 1754 copies/ml)(p=0.0178). CD4 count showed no correlation with AIN.
Conclusions: 1) The rate recommended by the Bethesda system of <5% ASCUS should be raised in the examination of anal cytological samples. High rate of HPV-positive ASCUS cases and low ASC/SIL+ rate suggest a tendency to under call ASCUS. 2) High rate of ASCUS progression to SIL (50%) and of samples with negative cytology progressing to atypical findings (38.6%) with associated high risk HPV and increased HIV viral load suggests that using the Bethesda criteria on anal cytology results in under calling in negative and atypical cases. Modified cytologic criteria to increase the sensitivity of anal pap smears is suggested. Development of cytological standards for interpretation of anal cytology similar to the Bethesda system is necessary.

375 Management for Breast Cancer Patients with Clinically Negative Axilla in a Single/First Outpatient Visit: The Role of Aspiration Cytology in a Multidisciplinary Cancer Center

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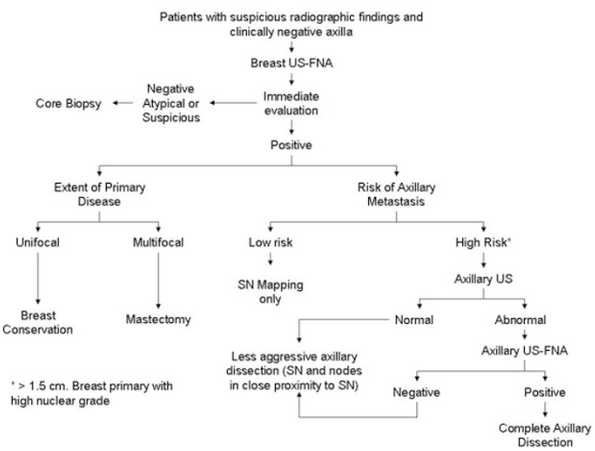
Background: The process of diagnosis and development of treatment plans for breast cancer patients currently involves multiple visits, often separated by lengthy time intervals that translate into heightened patient anxiety and increasing costs. With the efficient use of ultrasound guided fine needle aspiration biopsy (US-FNA), key features that affect the extent of surgery in the breast and axilla could be determined preoperatively in a relatively brief single outpatient visit.
Design: 235 patients were managed according to the following algorithm:



Results: No high risk (HR) patient required an additional visit for a breast primary diagnosis. Multifocality was suspected in 15% of the patients (n = 36) and proved by a positive (+) US-FNA in 5% (n = 12). Immediate breast reconstruction was not recommended in 35% of the patients (n = 81) in view of (+) axillary US-FNA and potential need for axillary radiation. Final nodal (N) stage for low risk patients (n = 29) was: N0 = 83%; N1mic = 10% and N1a with a single (+) node = 7%. N stage for HR patients with normal ultrasound or (-) US-FNA (n = 125) was: N0 = 71%; N1mic = 10%; N1a = 17%; N2-3 = 2%. Final N stage for HR patients with (+) US-FNA (n = 81) was: N0 = 0%; N1a = 44%; and N2-3 = 56%.
Conclusions: In institutions with expertise and experience in breast cytopathology, definitive diagnoses and treatment planning for most breast cancer patients can be achieved in a single outpatient encounter. Patients can be informed of their diagnoses, surgical planning and scheduling can be performed, and patient counseling can be initiated in a matter of hours. In addition to speed and accuracy, this algorithm is cost effective as it avoids unnecessary tissue biopsies and SN mapping procedures.

376 Cytologic Features of Meningiomas on Crush Preparations: Can Grading Be Accomplished?

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Background: Meningiomas are rarely subjected to aspiration, however, crush preparations (CP) are routinely examined in the frozen section suite. The goal of this study was to examine the cytologic features of meningiomas in CP and evaluate if benign meningioma (Grade 1), atypical meningioma (Grade 2) and malignant meningioma (Grade 3) can be diagnosed on CP.
Design: All cases of meningioma (1999-2007) which were submitted for frozen section at our institution were retrospectively reviewed. All cases were examined intra-operatively by frozen section and CP. The final histologic diagnosis was taken as the gold standard. CP slides were reviewed and cytologic features studied.
Results: 107 meningiomas cases were studied (92 Intracranial, 11 Spinal cord, 3 Orbital, 1 Olfactory groove). These included 72 (Grade 1), 22 (Grade 2) and 13 (Grade 3) cases. **Grade 1:** Hypercellular, syncitial large tissue fragments and single cells in a clean background. Tissue fragments were seen as clusters, whorls and sheets. Papillary configuration was noted in 29 cases. The cells were polygonal to spindle with eosinophilic, wispy cytoplasm. Nuclei were oval, sharply outlined and slightly eccentric with homogeneous even chromatin. Small centrally placed nucleoli were also present. Nuclear grooves and cytoplasmic pseudoinclusions were seen in 42 cases. The single cells were polygonal to spindle with wispy cytoplasmic prolongations, often with a fibrillary quality. A few of these single cells had hyperchromatic nuclei. Scattered naked nuclei were also seen in the background. None of the cells in all 72 cases had mitoses, karyorrhexis or necrosis.**Grade 2:** Hypercellular with a prominent sheet like architecture with whorls and clusters. The cells were polygonal with scant cytoplasm The cytoplasm was wispy and granular. The nuclei were slightly eccentric and showed slight pleomorphism. The nucleoli were central and large. Mitotic activity was seen in 2 cases. No evidence of necrosis was identified in any case. Fewer single cells as well as fewer naked nuclei as compared to Grade 1, were seen. Nuclear grooves as well as nuclear pseudoinclusions were seen in 12 cases.**Grade 3:** Hypercellular smears with pleomorphic clusters of cells as well as single cells. The cells had high nuceleus to cytoplasmic ratio and had prominent nucleoli. Frequent mitosis, hemorrhage and necrosis was present.
Conclusions: 1) Salient cytologic features of Grade 1-3 meningioma are reviewed. 2) It is difficult to seperate Grade 1 from Grade 2 meningioma on CP. 3) Grade 3 meningioma can be easily diagnosed on CP.

377 An Investigation of Radiologic and Cytologic Characteristics of Negative Endoscopic Ultrasound Guided Fine Needle Aspirations of the Pancreas

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Background: Pancreatic cancer usually has an aggressive clinical course with a 1.4 % overall 5 year survival. The development of endoscopic ultrasound (EUS) has facilitated the detection and sampling of small pancreatic lesions by fine needle aspiration (FNA) or core biopsies. The sensitivity and specificity of pancreatic EUS-FNA are reported to be 77-95% and 96-100%, respectively. Recent studies have observed that EUS-FNA is more sensitive than core biopsies in diagnosing pancreatic neoplasms. While the sensitivity of this procedure is well recognized, and is the basis for EUS-FNA's increasing clinical utilization, little is known about the characteristics of pancreatic lesions with false negative (FN) EUS-FNA results. Therefore, the current study attempts to investigate the cytologic and radiologic characteristics of EUS-FNAs with FN interpretation as determined by clinical follow up.
Design: EUS-FNA cases with a "negative for malignancy" cytologic diagnosis were identified retrospectively from the pathology department's files from 2000-2004. The cytologic slides were reviewed and clinical follow up information was obtained from the patients' medical records and/or by contacting patients. The patients were placed in 2 categories based on subsequent follow up information: no neoplastic process on follow up (NT) and pancreatic neoplastic process on follow up (T). Chi Square test was used for statistical analysis. A p value of ≤0.05 was considered statistically significant.
Results: Follow-up information was available in 51 cases: NT (35), T (16: 11 adenocarcinoma, 4 IPMN, 1 MCN). Analysis of collected data showed no statistically significant difference between T and NT groups with respect to lesion size, location, EUS findings or cyst fluid markers. NT group showed more inflammation compared to T patients (p=0.045). The negative predictive value (NPV) was 68.6%.
Conclusions: The size, location, and ultrasonographic findings of the target lesion are not significantly different in the T and NT EUS-FNA samples. While inflammation is more commonly seen in NT patients, additional parameters need to be assessed to determine their impact on the EUS-FNA NPV.

378 Visual Estimates of Nucleus-to-Nucleus Ratios: Can We Trust Our Eyes To Use the ASC-US and LSIL Criteria?

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Background: LSIL and ASC-US cells are characterized by nuclear enlargement of 2.5-3x and >3x the area of a normal intermediate squamous cell nucleus, respectively. We devised an experiment to determine the abilities of observers with various degrees of experience to estimate the area ratio between two nuclei.
Design: 45 participants (9 students, 18 residents, 8 cytotechnologists, 10 pathologists) judged the ratio of areas between the nuclei of two cells imaged at 100x objective magnification and projected on a screen. For test 1 (T1), 15 sets of two cells with previously determined nuclear areas were projected and the participants chose between 5 preset area ratios (1.0-1.4x, 1.5-1.9x, 2.0-2.4x, 2.5-2.9x, 3.0-3.4x). We then made 5 sets of schematic cells. The ratios between the nuclei of all cells used in the experiment (real and schematic) fell in the middle of the intervals (1.25x, 1.75x, 2.25x, 2.75x, 3.25x). A week after T1, the schematic cells were projected and their actual nuclear ratios were shown to the participants of test 2 (T2). After presenting the schematic cells, the original 15 sets of real cells were projected and judged. We recorded gender, medical function and individual cytologic experience.
Results: The kappa for all participants were .30 and .39 for T1 and T2, respectively. There were no significant differences in responses with respect to gender or medical function or to years of experience. The T1 and T2 responses were nearly identical for intervals corresponding to ASC-US and LSIL (2.75x and 3.25x, respectively).

	Correct Ratio	1.25x	1.75x	2.25x	2.75x	3.25x
T1 (N=42)	Mean	1.34	1.82	2.10	2.84	2.82
	Std Dev	0.19	0.49	0.44	0.42	0.41
	%Δ	+7.15%	+3.72%	-6.50%	+3.43%	-13.24%
T2 (N=33)	Mean	1.40	2.05	2.32	3.02	3.09
	Std Dev	0.24	0.50	0.39	0.34	0.28
	%Δ	+12.12%	+17.20%	+2.92%	+9.73%	-4.82%

Conclusions: This experiment suggests that experience does not significantly influence ability to judge the ratio of nuclear areas, but a slight improvement was seen after an instructional tutorial. Participants had the most difficulty in accurately judging the ratio of nuclei in the range of ASC-US and LSIL criteria. This may explain the well-known substantial variability in ASC-US and ASC/SIL ratios amongst cytotechologists and pathologists.

379 Are Follow-Up Thyroid Aspirations Necessary after a Single Fine Needle Aspiration (FNA) Diagnosis of Benign Nodule?

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Background: Thyroid fine needle aspiration (FNA) is widely considered to be a good diagnostic tool to assess thyroid nodules. Although most FNAs yield a benign diagnosis, there remains the question of whether or not to repeat the FNA in the case of a benign nodule. Recently published national clinical endocrinology guidelines of thyroid nodule management note there is no consensus to support whether to repeat an FNA at intervals if there is a benign diagnosis on the first FNA. We wished to restrospectively review our institution's data with regard to followup of patients whose aspirates yielded a diagnosis of benign nodule and to determine if there is an ideal number of follow-up FNAs.

Design: All patients that underwent thyroid FNA from January 1999 to December 2001 at a single institution were studied and followup information was obtained. During this period a total of 505 patients were examined. All FNAs were performed with cytopathologist-directed immediate adequacy assessment.

Results: 362/505 patients (72%) had an initial diagnosis of benign nodule. Average followup time for these 362 patients was 4.5 years. Out of the 362 patients, 115 had at least one repeat FNA and 78 had surgery. On chart review, the indications for surgery were either large goiter or a strong clinical suspicion of malignancy in spite of a benign FNA diagnosis. Of those with a repeat FNA, the diagnosis was follicular neoplasm in 1 patient (lost to follow up), papillary carcinoma in 2 patients (both subsequently having surgery), and benign in the remaining 112 patients. Of those having surgery, a benign nodule was found in 64 cases, and a malignancy in 14 cases. Of the malignancies, 5 were incidental papillary microcarcinomas. The remaining 9 clinically significant tumors were: papillary carcinoma (6), follicular lymphoma (1), metastatic renal cell carcinoma (1) and infarcted malignant neoplasm (1). On retrospective review of these 9 false negative cases, 7 were considered interpretive errors and 2 were sampling errors.

Conclusions: Excluding papillary microcarcinomas which were considered to be incidental, 9 malignancies were found out of the 362 initial aspirates diagnosed as benign nodule. On followup, our study found that after an initial FNA diagnosis of benign thyroid nodule, repeat FNA was only beneficial for 2 out of 115 patients. Our data suggest that a single FNA yielding a diagnosis of a benign thyroid nodule by FNA may be adequate unless there is strong clinical evidence of malignancy.

380 The Cytologic Diagnosis of Malignancy in Effusions Is Related to Sample Volume

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Background: Diagnostic challenges arise with low volume body cavity fluids by limiting sample cellularity, the number of cytopreparations that may be produced and ability to perform ancillary studies. The aim of this study was to investigate the relationship between body cavity fluid volume and the rate of suspicious and malignant cytologic diagnoses.

Design: Over a 3 year period, 2,630 body cavity fluids (1,389 pleural, 123 pericardial and 1,118 peritoneal fluids) had been received fresh and were processed into a single ThinPrep slide for cytologic diagnosis. A review of these cases was conducted categorizing the fluid samples into 6 volume classes based on the volume of sample received; <5 mL, 5 to <10 mL, 10 to <20 mL, 20 to <40 mL, 40 to <100 mL and 100 mL or more. For each volume class, the frequency of a suspicious or malignant cytologic diagnoses was noted in addition to the gross appearance of the fluid, patient age and gender. The fluid classes were then compared considering all fluid types together as well as evaluating pleural and peritoneal fluids separately.

Results: The rate of malignant cytologic diagnoses increased with increasing fluid volume from 15.6% in fluids <5 mL to 32.4% in fluids >100 mL. The rate of suspicious cytologic diagnoses also increased with increasing fluid volume, from 1.6% in fluids <5 mL to 2.6% in fluids >100 mL. The same relationship between fluid volume and the rate of suspicious or malignant cytologic diagnoses was seen for all fluids combined as when pleural and peritoneal fluids were considered separately. This same relationship was also evident in both men and women, although women had a higher frequency of malignant diagnosis in all volume classes. The mean age was not significantly different among the different volume classes and there was a trend for the smaller volume samples to be described as clear with the larger volume samples to appear bloody.

Conclusions: The rate of malignant diagnoses for low volume fluid samples (<5 mL) is less than one-half the rate of malignant diagnoses for large volume fluids (>100 mL), with the rate of malignant diagnosis increasing with increasing fluid volume. This relationship was evident when all fluids were combined, as well as for pleural and peritoneal fluids when considered separately and appeared to be independent of patient age and gender. It is possible that the small volume of fluid received for cytologic evaluation is limiting the cytologic detection of malignancy.

381 Does Biomarker Analysis of Endoscopic Ultrasound Guided Fine Needle Aspiration Biopsy (EUS-FNAB) Material Correlate with Cytologic Interpretation?

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Background: It has been proposed that a limited panel of biomarkers [CEA levels, KRAS mutation, loss of heterozygosity (LOH)] may be used to classify pancreatic cystic lesions, but this hypothesis has not been independently validated. The aim of this study was to determine whether biomarker analysis of cyst fluid is a useful adjunct to the cytologic evaluation of EUS-FNAB material from pancreatic cystic lesions.

Design: 10 EUS-FNAB samples obtained from pancreatic cystic lesions were tested at a reference laboratory for CEA, KRAS-2 activating mutations (exon 1) and LOH at 7 microsatellite markers linked to tumor suppressor genes. The lesions were categorized as mucinous (MC) or non-mucinous (NMC) cysts based on the presence, or absence, of KRAS-2 mutations according to an algorithm set by the laboratory. MCs with LOH at 2 loci, or elevated CEA, were considered to be severely dysplastic. NMCs were classified as serous cystadenomas (SCA) or reactive/indolent based on the presence, or absence, of LOH. Cytology specimens from each case were retrospectively reviewed in a blinded fashion and classified as non-neoplastic, SCA, or MC with mild or moderate/severe dysplasia.

Results: Biomarker and cytologic evaluations yielded concordant diagnoses in only 3 (30%) cases (1 SCA, 2 MCs with mild dysplasia). Five cases interpreted as reactive/indolent based upon biomarker studies were cytologically classified as SCAs (2 cases) and MCs with mild (2 cases) and moderate/severe (1 case) dysplasia. Both SCAs were stable upon follow-up imaging and 2 MCs were surgically resected (mucinous cystadenoma with mild dysplasia and intraductal papillary mucinous neoplasm with moderate dysplasia), confirming the diagnoses. Finally, biomarker analysis yielded diagnoses of MC with mild and severe dysplasia in 1 case each, whereas the cytologic impressions were non-neoplastic and MC with mild dysplasia, respectively.

Conclusions: There is a high (70%) discordance between the pathologic diagnosis and interpretation of biomarker studies performed on pancreatic cyst contents. We believe that, although biomarker analysis may ultimately prove to be a useful tool, the current panel utilized by some commercial laboratories is not reproducibly accurate in the evaluation of pancreatic cystic lesions.

382 Use of the ThinPrep Imaging System Does Not Result in Higher ASC-US Rates or Subsequent Referrals to Colposcopy

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Background: Use of the ThinPrep Imaging System (TIS) (Cytyc, Boxborough, MA) increases Pap test sensitivity for LSIL and HSIL detection (Miller *et al*., Diagn Cytopathol 2007;35:213). The effect of TIS on ASC-US remains unclear. Herein, we sought to determine whether TIS has altered ASC-US rates in our institution compared to manual screening (MS) and whether this has increased the number of women referred for colposcopy.

Design: A computerized search for all ASC-US cases with reflex Human Papillomavirus (R-HPV) testing over a 6 month period (1/1/07 to 6/30/07) was conducted. We determined which were screened by cytotechnologists (CT) utilizing TIS and by MS alone. The TIS was in clinical use for 16 months prior to the study. Limitations in the volume of slides TIS can screen account for the cases in the MS arm. Testing for R-HPV was performed by the Hybrid Capture II test (Digene, Gaithersburg, MD). The cases were screened by 12 senior-level CT and signed out by 4 cytopathologists. The ASC:SIL ratio for the lab was 1.3 during the previous year (2006). Pertinent histological follow-up, when available, was also determined.

Results:

Table 1: The Effect of TIS on ASC-US and High-Risk R-HPV Positivity Rates

Screening	Total	ASC-US		High-Risk R-HPV Positive		
		Number	% of Total	Number	% of ASC-US	% of Total
TIS	11093	489	4.41	243	49.7	2.19
MS	22532	948	4.21	500	52.7	2.22

Of 743 women with ASC-US and positive R-HPV, 262 had follow-up biopsies (TIS screened: 83; MS: 179). Among the 83 TIS screened, the biopsies found: 30 CIN 1 (36%), 10 CIN 2 (12%), and 1 AIS (1%); for the 179 MS, the biopsies found: 71 CIN 1 (40%) and 21 CIN 2-3 (12%).

Conclusions: 1) There was no statistically sigificant difference between the ASC-US rate on TIS and MS Pap tests (4.41% vs 4.21%)(p=0.13). 2) The R-HPV positivity rate was also not statistically significantly different, as either a share of ASC-US (49.7% vs 52.7%)(p=0.064) or of the entire population (2.19% vs 2.22%)(p=0.76). 3) As a result, TIS screening does not lead to increased colposcopic referrals.

383 Routine Review of Liquid-Based Papanicolaou Test Slides in Women Aged 30 Years or More with Positive Adjunctive HPV Testing Following an Interpretation of NILM: A New Quality Assurance Measure

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Background: The Food and Drug Administration has recently approved the use of adjunctive Human Papillomavirus (HPV) testing in liquid-based Pap tests (LBPT) in women over age 30 with negative cytology. Women with a NILM diagnosis who test negative for high-risk HPV are eligible for reduced frequency of future LBPT screening. We have analyzed the characteristics of the small subset who test positive for high-risk HPV.

Design: We used a computerized search to identify all women with a LBPT diagnosis of NILM who had adjunctive HPV testing ordered by the requesting physician over a 6 month period. We compiled the subsequent HPV results as determined by the Hybrid Capture II technique (Digene, Gaithersburg, MD). The LBPT were screened by 12 senior

cytotechnologists (CT). Cases determined to be negative by the CT were signed out directly, with the remainder referred to 4 cytopathologists for review. All slides from high-risk HPV positive cases were reviewed by a consensus conference of 1 senior cytotechnologist, 1 cytopathology fellow, and 2 cytopathologists. The follow-up biopsy results for these patients were also retrieved.
Results: During the study period, 1/1/06-6/30/06, 32147 LBPT were screened. Of these, 1132 (3.52%) were signed-out as NILM and sent for adjunctive HPV testing in women aged 30 years or more. Of the 1132 cases sent, only 45 (3.98%) were high-risk HPV positive. Nine of these had biopsy follow-up: 4 were negative, 3 showed CIN 1, and 2 showed CIN 2. One of the women with CIN 2 on biopsy had a cervical conization procedure which revealed CIN 3. Review of the 45 slides led to revised Pap test interpretations in 5 cases (11.1%): 4 were upgraded to ASC-US and 1 to ASC-H by consensus. The slide re-interpreted as ASC-H corresponded to the patient later found to have CIN 3.
Conclusions: Adjunctive HPV testing in women over 30 is new and increasingly popular. The vast majority of women tested are high-risk HPV negative, but rare cases are positive. A substantial proportion of cases found to have high-risk HPV show cellular atypia on the corresponding LBPT slides upon later review. We suggest routine review of all slides signed out as NILM in women found to be positive for high-risk HPV on adjunctive testing as a new quality assurance measure.

384 Detection of High Risk HPV Using Invader HPV Analyte Specific Reagents (ASR) vers 1.0

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Background: The American College of Obstetricians and Gynecologists recommends cervical cancer screening for human papillomavirus in patients with ASCUS. The results of HPV DNA screening tests guide future treatment and management in these patients. We evaluated the Invader HPV Analyte Specific Reagents (ASR) version 1.0 relative to the FDA approved Hybrid Capture® 2 High-Risk HPV DNA Test™ (HC2) in detecting DNA from high risk HPV types.
Design: 119 randomly chosen cervical cytologic specimens were tested for high risk HPV DNA using Invader. This set includes 85 (60 ASCUS and 25 negative by cytology) sent out for HC2 testing per clinician request, 27 negative on cytology (not sent for HC2 testing), and 7 previously diagnosed as HSIL used as an internal control. The results for all clinical samples tested with Invader were evaluated in the context of cytology findings and, if conducted, the corresponding results obtained using the HC2 test. Discrepant results between the Invader and the HC2 test were resolved using consensus PCR and DNA sequencing.
Results: All 7 samples with a cytologic diagnosis of HSIL tested positive for DNA from high risk HPV types using the Invader ASR. Among the 27 samples with negative cytology, 2 samples tested positive by Invader and were confirmed to have high risk HPV by consensus PCR and DNA sequencing. Of the remaining 85 samples, 73 (86%) (54 ASCUS and 19 negative) demonstrated concordant results between the Invader HPV ASR version 1.0 and the HC2 HPV DNA methods. Of the 12 with discordant results, 11 samples were HC2 positive/Invader negative with 7 of these confirmed negative for high risk HPV DNA by consensus PCR and DNA sequencing. 4 samples tested positive by HC2, negative by Invader, and sequenced positive for high risk HPV; however, one of the negative results by Invader was not valid due to low genomic DNA levels. 1 sample was negative with the HC2 method and positive with the Invader assay; this patient had a history of LSIL.
Conclusions: In summary, the Invader HPV ASR is robust, easy to use, demonstrates good concordance with HC2, may be more specific, and may be an attractive methodology for the laboratory diagnosis of high risk HPV.

385 Independent Diagnostic Accuracy of Flow Cytometry of Fine Needle Aspirates: A 10 Year Experience with 511 Cases

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Background: Fine needle aspiration (FNA) with flow cytometry (FC) is common practice for the evaluation of lymphoproliferative disorders, but is not without controversy. Although there is information regarding the diagnostic accuracy of combined FNA with FC, there is no large scale study devoted to independently analyzing FC of these specimens. Since FC is often performed and interpreted by a non-cytopathologist (sometimes at a different institution), knowledge of the independent performance characteristics of FC would be useful to the cytopathologist correlating FC results with cytomorphology.
Design: 511 aspirates from 487 patients sent for FC from 1997-2006 were identified. FC reports were retrospectively reviewed and the final diagnosis for FC was categorized as monoclonal/"consistent with lymphoma" (M; 44.4%), atypical/"suspicious for or suggestive of lymphoma" (A; 6.3%), normal or reactive (N; 45.4%), or insufficient cellularity for analysis (I; 3.9%). Also, studies with a limited panel (<6 antibodies) were noted (97 of 491; 19.8%). Abnormal immunophenotype (M + A) by FC was considered a positive test. The gold standard was established by patient follow up with subsequent excisional biopsy in 65.5%, treatment based on FNA + FC diagnosis in 15.7%, and clinical course in 18.8%. Clinical follow up of greater than 6 months was required for a negative flow cytometry without subsequent biopsy (median follow up 34 months). Adequate follow up to establish a gold standard diagnosis was obtained in 92.1% (452 of 491).
Results: The diagnostic accuracy of FC was 88.7% (95% CI of 85.5-91.3%) with a sensitivity of 85.2% and specificity of 94.6%. Of the 452 specimens, there were 242 true positives (53.5%), 159 true negatives (35.2%), 42 false negatives (9.3%), and 9 false positives (2.0%). 20.9% of the negative FC were falsely negative, and 66.7% of these had abnormal cytomorphology. The diagnostic accuracy was lower for specimens with a limited panel (82.0%) as compared to non-limited specimens (90.4%; p-value of 0.038).
Conclusions: 1. FC is an accurate ancillary test in the evaluation of atypical lymphoid aspirates. 2. There is a relatively high false negative rate for FC, thus abnormal cytomorphology should trigger subsequent tissue biopsy, even in the face of negative FC. 3. There is a low, but real, false positive rate for FC; therefore, FNA with low suspicion or reactive appearing cytomorphology and abnormal immunophenotype require further characterization rather than basing lymphoma treatment solely on FC results.

386 The Value of Touch Imprint Cytology in Endoscopic Ultrasound Guided Trucut Biopsy

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Background: Endoscopic ultrasound (EUS) guided Trucut biopsy (TCB) is a recent technique that enables acquisition of tissue cores for histologic assessment. Touch imprint cytology (TIC) can be performed at the time of biopsy to assess the adequacy of the sample; however, limited information is available on the diagnostic value of TIC of these specimens. We propose that TIC can provide an accurate and rapid diagnosis when used with EUS guided TCB. The objective of this study is to investigate the diagnostic accuracy of TIC compared with TCB and of both methods combined.
Design: Consecutive EUS guided TCB with TIC (n=109) were retrospectively and independently reviewed by a surgical pathologist (for TCB) and cytopathologist (for TIC) blinded to the final diagnoses. There was no limitation to site or indication for biopsy; included were 43 lymph nodes, 24 pancreatic masses, 17 lung/mediastinal masses, 16 tumors of the gastrointestinal tract, and 9 intra-abdominal tumors. Follow-up information was obtained to establish a gold standard final diagnosis (subsequent surgical resection of the lesion, n= 39; chemotherapy and/or radiotherapy based on TCB+TIC diagnosis, n= 36; clinical impression and course, n= 34). Diagnostic accuracy of TCB alone, TIC alone, and combined TCB+TIC were calculated and correlation between TCB and TIC was evaluated.
Results: The diagnostic accuracy of TCB was 92.7% (95% CI of 83.1-97.3%), TIC was 82.6% (95% CI of 74.3-88.6%), and TCB+TIC was 95.4% (95% CI of 89.4-98.3%). When comparing the two methods, the diagnostic accuracy of TCB alone was superior to TIC alone (p-value = 0.038); TCB was diagnostic in 14 cases that were nondiagnostic by TIC. Although the increased diagnostic accuracy of combined TCB+TIC over TCB alone did not reach statistical significance (p-value = 0.57), the addition of TIC allowed for the identification of 3 (2.8%) cases of malignancy that were not identified on TCB alone. In 22 cases TIC was considered diagnostic, but TCB provided additional specific diagnostic information (further characterization of 11 spindle cell lesions, 5 primary sites of malignancy, 4 lymphomas, and 2 granulomatous lymphadenitides).
Conclusions: TIC is a valuable tool for use in EUS guided TCB; TIC is independently diagnostically accurate allowing for confidence in a rapid preliminary diagnosis, and it provides additional diagnostic value when combined with TCB.

387 Assessment of a Fluorescence In Situ Hybridization (FISH) Reflex Test for Bronchial Brushing Specimens Evaluated by Routine Cytology

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Background: Our laboratory performs FISH reflex testing for the detection of lung carcinoma on bronchial brushing specimens with an equivocal or negative cytology diagnosis. The purpose of this study was to assess the performance of this FISH reflex test in routine clinical practice.
Design: From June 2006 to August 2007, 210 bronchial brushing specimens were submitted for cytology and FISH reflex testing. FISH slides were hybridized with the LaVysion™ (Abbott Molecular Inc. Des Plaines, IL) probe set when the cytology diagnosis was negative or equivocal. Specimens were considered positive by FISH when ≥5 cells showed gains of two or more loci/chromosomes (polysomy) or when ≥10 cells exhibited 4 copies of each locus/chromosome (tetrasomy). A positive bronchoscopic biopsy, surgical resection or transbronchial needle aspiration obtained within one year of the original cytology result was considered evidence of malignancy (gold-standard). Twenty-six specimens were excluded due to the lack of a follow-up pathology result.
Results:

Comparison of Cytology and FISH Reflex

	Sensitivity	Specificity	NPV	PPV
Cytology Only*	35% (34/97)	100% (87/87)	58% (87/150)	100% (34/34)
Cytology Only#	53% (51/97)	86% (75/87)	62% (75/121)	81% (51/63)
Cytology+FISH Reflex*	73% (71/97)	78% (68/87)	72% (68/94)	79% (71/90)

*Equivocal cytology as negative; #Equivocal cytology as positive

Routine cytology diagnosed 34 specimens as positive, 29 as equivocal and 121 as negative. Of these 184 patients, 97 (53%) were diagnosed with lung carcinoma. Cytology with FISH reflex had significantly higher ($p<0.001$) sensitivity than cytology alone when equivocal cytology diagnoses were considered positive (73% vs. 53%) or negative (73% vs. 35%), but also had lower specificity. Of the 150 specimens reflexed for FISH, 56 specimens (37%) were diagnosed with an abnormality. Thirty of the 41 (73%) specimens diagnosed as polysomy and 7/15 (47%) with tetrasomy had lung carcinoma, while 26/94 (28%) specimens diagnosed as negative by FISH had lung cancer.
Conclusions: These data suggest that routine cytology with FISH reflex is significantly more sensitive than cytology alone for the detection of lung carcinoma. Furthermore, patients diagnosed with a FISH abnormality may be at higher risk of having lung carcinoma than those with a negative FISH result. Longer clinical follow-up is needed to further evaluate these results.

388 Effects of Dilution of Residual PreservCyt Solution on Hybrid Capture Results

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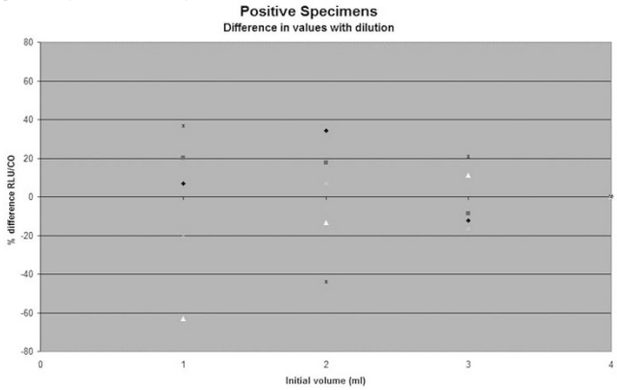
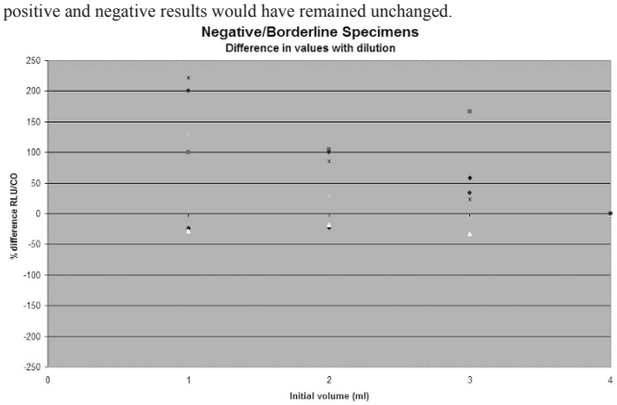
Background: Reflex testing for high risk human papillomavirus (hrHPV) of residual solution from ThinPrep Pap Tests (Cytyc, Marlborough, MA) with an interpretation of ASC-US is a common practice. The Hybrid Capture 2 assay (hc2, Digene Corporation, Gaithersburg, MD) requires 4 milliliters (mls) of solution for testing. Over 5% of specimens in our laboratory do not have sufficient volume and therefore cannot be tested. Dilutional studies are a standard methodology in the clinical laboratory. This study assesses the results of dilutional studies using the hc2 assay.

Design: Residual specimen from 11 ThinPrep Pap Tests for which hc2 results were known were selected. The original relative light unit/cutoff (RLU/CO) values ranged from 0.12-2540.97. Aliquots of 3.0, 2.0, and 1.0 mls were removed from each, and all were brought to a total volume of 4.0 ml using PreservCyt solution. hc2 testing was performed on all reconstituted specimens and RLU/CO values were recorded. A calculated RLU/CO value (cRLU) was determined for each aliquot and compared to the original, undiluted value. The difference between each cRLU and original RLU/CO was calculated, with results compared as percentages.

Results: Table 1 shows results from specimens with hrHPV detected. The cRLU range was - 63% to + 37% (mean, -1.6%, SD, 27.8). All cRLUs would have been considered positive (hrHPV detected).



For borderline positive and negative (not detected) results (Table 2), cRLU range was -32 to +221% of original values (mean, 62.3%, SD 80.9). Interpretation of borderline positive and negative results would have remained unchanged.



Conclusions: Reconstitution of residual PreservCyt solution shows wide variation in calculated results, exceeding allowable limits for normal control results. However, all interpretations remained unchanged. This method may be effective, especially with positive results, in decreasing quantity not sufficient rates on reflex testing.

389 Axillary Ultrasound Combined with Fine Needle Aspiration Predicts Final Lymph Node Stage in Breast Cancer Patients with Clinically Negative Axillae but at High Risk for Metastasis

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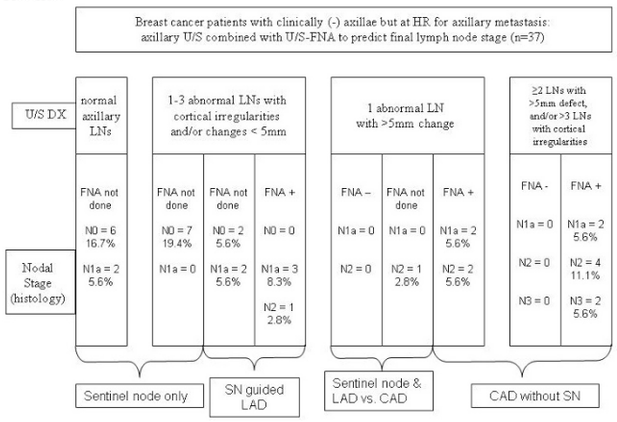
Background: In breast cancer, axillary nodal status is the most powerful predictor of survival. For patients without palpable axillary disease, sentinel node (SN) mapping and axillary dissection are standard of care. Previous studies have demonstrated the utility of sonographic (U/S) axillary imaging to detect nonpalpable metastases. These studies showed that patients with minimal nodal changes on ultrasound and negative (-) axillary fine needle aspiration (FNA) require only SN mapping to evaluate their axillae, and might benefit from limited axillary dissections (LAD) when nodal disease was restricted to microscopic tumor deposits in a single node. Others with significantly abnormal axillary U/S and positive FNA can proceed directly to complete axillary dissection (CAD). This prospective study examines the ability of axillary U/S combined with U/S-FNA of high risk (HR*) breast cancer patients with clinically (-) axillae to predict the number and size of axillary LN metastases, and therefore final LN stage.

Design: Patients with clinically (-) axillae who met HR criteria for metastases were prospectively identified. These 36 patients underwent U/S examination of their axillae, and the number, size, and characteristics of abnormal LNs were recorded. When significant LN alterations were present, U/S-FNA was performed. The combined U/S findings and FNA results were used to predict the LN stage and the type of axillary resection best suited according to extent of disease. Predictions were compared to the number and size of metastases and the final LN stage at surgical resection. *HR criteria: patients with grade III >1 cm and grade II > 1.5 cm. (dimensions determined by imaging).

Results:



Conclusions: This multi-disciplinary approach can reliably stratify patients pre-operatively into those requiring CAD, forgoing SN mapping, those who may required SN mapping alone, and patients that may benefit from SN guided LAD.

390 Fibrin-Like Material in SurePath® Liquid-Based Pap Tests: Characteristics, Incidence, and Clinical Significance

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Background: When interpreting liquid-based Pap tests, it is not uncommon to come across unknown material or artifacts. While this material at times evokes curiosity, it is often ignored to concentrate on the more important task of identifying cervical dysplasia. In the present study, we investigated the incidence and potential clinical setting of a thick stringy and bubbly ″stretched taffy-like″ eosinophilic material present in SurePath® liquid-based Pap tests believed to represent fibrin clot, which is morphologically distinct from tumor diathesis.

Design: In the calendar year 2005, 539 consecutive SurePath® liquid-based Pap tests were retrospectively reviewed for the presence of fibrin clot. Of these, 33 (6%) Paps were found to have this material, representing the study set. The patient's age, Pap test interpretation, last menstrual cycle, and pregnancy status were all recorded. In addition, several prospective cases with this identified material were analysized with immunohistochemical and cytochemical stains to determine its makeup and characteristics.

Results: The patient's average age was 30 years (range,16-62 years). A majority (91%) of the cases were interpreted as NILM. Candida and shift in bacterial flora were found in 5 and 4 cases, respectively. In relation to menstrual cycle, 6 patients were day 1-13 and 12 were day 14 or greater. Two patients were postmenopausal. Seven patients were pregnant (p = 0.021, Fisher exact). The material demonstrated strong immunohistochemical staining for factor VIII, supporting fibrin clot origin.

Conclusions: The presence of fibrin clot material is seen in a small but significant number of SurePath® liquid-based Pap tests. While its presence does not appear to be associated with menstruation or abnormal Pap findings, there is a statistically significant correlation with pregnancy. Further studies examining additional cases, as well as, looking into pregnancy outcomes will be investigated for any potential clinical significance. None of the patients in this retrospective study had adverse pregnancy outcomes. The material does not appear to be of clinical significance and should not be mistaken for tumor diathesis. Since this material is fibrinated, it is probably not due to blood from microtrauma caused by the Pap procedure.

391 Evidence-Based Guidelines for the Optimization of Immunostain Panels in Pleural Effusions with Malignant Cells

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Background: Pleural effusions with malignant cells are frequently studied with immunohistochemistry (IHC) to identify the tumor cell origin. There is no established methodology to help select cost effective IHC panels. This study aims to develop evidence-based guidelines for the use of IHC panels for the diagnosis of malignant pleural effusions.

Design: The use of IHC in 243 pleural fluid cytologies with malignant epithelioid cells was reviewed. This cohort was selected from 1360 pleural effusions evaluated in our laboratory from 2005-2007. Tumor cell origins included malignant mesothelioma and carcinomas of lung, breast, and other origins. In the absence of standards, variable IHC panels had been selected from a menu of 31 antibodies. Pre-test and post-test odds of each antibody being reactive were calculated by tumor cell origin site. The probability and odds of each antibody as well as probability ratios (PRs) and odds ratios (ORs) of various combinations of antibodies were calculated to help select cost effective panels.

Results: The average number of antibodies used in the original evaluation of the cases was six, but some cases had been worked up with up to 22 antibodies. The most common antibodies used were TTF-1, MOC31, Ber-EP4, CK7, CK20 and CEA. Not surprisingly,

the majority of IHC tests did not contribute to tumor cell origin identification. Calretinin and Ber-EP4 provided the best probabilities and PRs to distinguish mesotheliomas from carcinomas. They yielded lower PRs for the diagnosis of tumor cell origin of Müllerian origin than for others. TTF-1 and either ER for females or PSA for males provided the best PRs, odds and ORs to distinguish lung from breast, Müllerian or prostatic neoplasms. A five antibody panel using calretinin, Ber-EP4, TTF-1, CDX-2 and ER or PSA provided the best probabilities, PRs, odds and ORs for all possible diagnoses of tumor cell origin.

Conclusions: The use of Bayesian statistics such as probabilities, odds, PRs and ORs provides an effective tool for the development of evidence-based guidelines for the optimization of IHC panels in cytopathology.

392 Causes of Discrepancies between Reflex HPV Tests and Subsequent Biopsy Results

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Background: HPV DNA reflex testing is currently used in most institutions to triage women with a diagnosis of ASC-US for colposcopy. Studies have shown that the rate of HPV-positivity and the rate of underlying dysplasia decline with age in women with ASC-US. The aim of this study was to assess if the lower rate of dysplasia following a diagnosis of ASC-US in women over 50 is due solely to the lower rate of underlying HPV infection or if a change in the relative proportion of HPV genotypes is a contributing factor.

Design: Searches through the molecular diagnostic, cytopathology and surgical pathology databases of a large health care system were performed and all cases of CIN2/3 preceded by a hrHPV(-) ASC-US diagnosis were identified. Control groups consisting of CIN2/3 preceded by a hrHPV(+) ASC-US diagnosis were also identified. A group with no CIN preceded by a hrHPV(+) ASC-US diagnosis was randomly selected. A PCR-based HPV DNA detection technique was used. All biopsies and LEEP/cones were reviewed blinded to the results of the preceding HPV test. Longitudinal extent of the lesion was categorized: focal (1mm), intermediate (1-3mm), extensive (>3mm). p16 and Ki67 stains were performed on all sections; cases were considered negative for these markers if they lacked diffuse intense nuclear and cytoplasmic p16 staining or significant suprabasal nuclear staining with Ki67. The corresponding LBPT were reviewed, and the overall cellularity and number of abnormal cells were estimated.

Results: In the hrHPV(-) CIN 2/3 group, p16 and Ki67 were positive in 31/33 cases (94%). Positivity was not significantly different in the hrHPV(+) CIN 2/3 group (95%). This suggests that histological overdiagnosis is minimal. The mean age of women with hrHPV(-) CIN 2/3 was 35 years and 25 years for those with hrHPV(+) CIN 2/3. All of the hrHPV(-) CIN 2/3 cases had a satisfactory pap with a cellularity range of 10,000-100,000 and mean cellularity of 30,000. The extent of dysplasia was greater in hrHPV(+) CIN 2/3 (28%) than in hrHPV(-) CIN 2/3 (12.9%). Of the 18 hrHPV(+) cases with follow-up biopsies negative for dysplasia, 3/18 cases (17%) were positive by immunostains.

Conclusions: We found that negative hrHPV test preceding a diagnosis of CIN2/3 is only rarely due to histologic overdiagnosis, but appear to be due to focality of disease and increased age of patient. In addition to deeper sections staining for p16 and Ki67 may be helpful in finding additional cases of CIN2/3 in biopsies following a hrHPV+ case.

393 Human Papillomavirus in Atypical Squamous Cervical Cytology: The Invader HPV Test as a New Screening Assay

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Background: In surveillance for cervical neoplasia, cytologically atypical cells of undetermined significance (ASCUS) present a significant clinical issue, often dependent on human papillomavirus (HPV) testing for triage of patients. HPV types 16 and 18 appear to be the greatest concern with HPV16 being associated with a 33% two year risk for CIN3. The Invader HPV test (Inv2) by Third Wave Technologies, Inc. is a recently developed set of analyte specific reagents that employs probe sets for detection of 14 high risk HPV subtypes, grouped as A5/A6 (HPV 51,56,66), A7 (HPV 18,39,45,59,68) and A9 (HPV 16,31,33,35,52,58). This report describes performance characteristics of the Inv2 test in screening ASCUS cervical cytology specimens with correlation to the results of the Hybrid Capture II HPV test Probe B (HC2) by Digene.

Design: 94 consecutive ASCUS samples and 39 negative cytology samples were HPV typed with Inv2. Results were correlated with HC2 with Roche PCR Linear array employed as a reference method for discordant results.

Results: The concordance rate for ASCUS cases between Inv2 and HC2 was 86.8%. The analytical positive and negative predictive values for Inv2 were 94% and 97% respectively. There were 12 discordant ASCUS cases: 7 Inv2 negative/HC2 positive specimens and 5 Inv2 positive/HC2 negative specimens (Table 1). There were 5 discordant negative cases: 4 Inv2 positive/HC2 negative specimens and 1 Inv2 negative/HC2 positive specimen (Table 2). Linear array of discordant cases detected 2 cases of false negatives for HPV type 16, one each by Inv2 and HC2.

Table 1: Correlation of HPV Results obtained by Invader 2 and Hybrid Capture 2 assays

		Invader 2 Results					
	Total Cases	Indeterminate	Total -	Total +	A9 Probe + (HPV 16)	A7 Probe + (HPV 18)	A9 & A7 + (HPV 16 & 18)
ASCUS Cytology							
HC2B -	48	3	40	5*	1	1	0
HC2B +	46	0	7*	39	19	9	3
Total	94	3	47	44	3	10	3
Negative Cytology							
HC2B -	37	3	30	4*	1	1	2
HC2B +	2	0	1*	1	1	0	0
Total	39	3	37	2	2	1	2

HC2B, Hybrid Capture probe B; *, dicordant cases

Table 2: Discordant HPV typing by HC2 and Inv2

	HC2B +	Inv2 +	PCR Linear Array High Risk HPV +
ASCUS (n= 7)	7	0	2
ASCUS (n = 5)	0	5	3
Negative (n=1)	1	0	0
Negative (n=2)	0	2	1

HC2B, Hybrid Capture 2 probe B; Inv2, Invader 2

Conclusions: Overall, performance of Inv2 was comparable to that of the HC2 assay. The Inv2 HPV test could provide a useful test for sub-grouping of HPV 16/18 with potential for improved risk stratification of patients with ASCUS results.

394 Invader Human Papillomavirus (HPV) 16 and 18 as an Adjunct to HPV Screening of Cervical Atypical Squamous Cells of Undetermined Significance (ASCUS)

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Background: Atypical squamous cells of undetermined significance (ASCUS) in cervical cytology is a significant clinical dilemma. With HPV 16 and 18 attributing to 70% of cervical carcinoma, HPV screening is becoming a standard of practice in ASCUS. We have recently demonstrated the clinical utility of the Invader HPV test (Inv2) as a robust HPV screening test for the detection of 14 high risk HPV types by pooled probe sets. While positivity for probe sets A9 or A7 correlate with HPV 16 or 18 infections respectively, other high risk HPV non-16/18 are detected in these two probe sets. True infection by HPV 16/18 remains uncertain after the initial Inv2 screening assay. The Inv2 type specific assay for HPV 16 and 18 are recently developed analyte specific reagents whose performance characteristics and applications as follow-up tests after the Inv2 screen have not been characterized.

Design: Thirty-seven ASCUS cervical cytology samples were HPV screened with Inv2 (Third Wave Technologies, Inc) probe sets A5/A6 (HPV 51,56,66), A7 (HPV 18,39,45,59,68), and A9 (HPV 16,31,33,35,52,58). These samples were subsequently HPV typed with Inv2 HPV 16/18 type specific assays.

Results: Nine of the 37 ASCUS specimens were Inv2 HPV 16 positive (Table 1). Eight of the 13 samples screened Inv2 A9 positive were Inv2 HPV 16 positive. All samples were negative for Inv2 HPV 18 including the 4 cases positive for Inv2 A7.

Table 1: Invader16/18 Assay as Compared with Invader Screening Assay in Cervical ASCUS

	Invader Screening Assay						
	A5/A6 +	A7 (HPV 18) +	A9 (HPV 16) +	Both A7 and A9 +	Indeter-minate	Negative	Total
Invader HPV 16	0	0	8	0	3	1	12
Invader HPV 18	0	0	0	0	0	0	0
Negative	4	4	5	1	0	11	25
Total	4	4	13	1	3	12	37

Conclusions: Sixty-two percent of female patients who screen Inv2 A9 positive are confirmed to have HPV 16 infection by the Inv2 HPV 16 probe specific assay. However, there remain a percentage of patients who are high risk HPV positive but are not 16/18. Due to the greater clinical urgency in patients infected with HPV 16/18, patients who screen positive for Inv2 A9 or A7 may benefit from type specific HPV 16/18 characterization by the Inv2 16/18 assays.

395 Criteria for the Cytologic Diagnosis of Papillary Breast Lesions: A Logistic Regression Analysis

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Background: The cytologic diagnosis of papillary lesions of the breast is challenging due to significant morphologic overlap with fibrocystic change, fibroadenomas and ductal carcinomas. Previously described criteria have emphasized the importance of papillary structures and macrophages. However, papillary fragments are often absent and macrophages are non-specific. In this study, we compared the cytologic features of biopsy-proven papillary lesions with those of non-papillary lesions, including a feature not previously described - degenerative cytoplasmic vacuoles.

Design: 82 cases (52 biopsy-confirmed papillary and 30 non-papillary) were obtained from the Cytopathology files at Stanford and UCSF. The papillary cases consisted of intraductal papillomas (22 FNA, 20 nipple discharge) and papillary carcinomas (10 FNA). The non-papillary cases included fibroadenomas (9), fibrocystic change (11), phyllodes tumor (1) and ductal carcinomas (9). Each case was scored for the presence or absence of the following cytologic features: Degenerative cytoplasmic vacuoles, proteinaceous background, paucity of myoepithelial cells, papillary structures with or without vessels, histiocytes (foamy or hemosiderin-laden), necrosis, metaplastic cytoplasm, columnar cells, single intact cells, mitotic figures, nuclear enlargement, nuclear membrane irregularities, nuclear pleomorphism, prominent nucleoli, and overall cellularity. Stepwise logistic regression was performed using SPSS statistics software.

Results: Four features were found to be predictive of a papillary lesion: Degenerative cytoplasmic vacuoles, proteinaceous background, absence of myoepithelial cells, and papillary structures with vessels. The regression coefficients of each of the four predictive variables within the logistic regression model were statistically significant ($p<0.008$). Using these four features, the model was able to predict 81.7% of the papillary breast lesions. However, the model was not able to predict benign vs. malignant papillary lesions.

Conclusions: Degenerative cytoplasmic vacuoles, a feature that has not been previously studied in papillary lesions of the breast, is identified by logistic regression analysis to be useful in distinguishing papillary lesions from non-papillary breast lesions. Overall, four features were found to be predictive of a papillary lesion: degenerative cytoplasmic vacuoles, papillary structures with vessels, proteinaceous background and paucity of myoepithelial cells.

396 The Significance of Cervical Parakeratosis in the False Negative Papanicolaou Smear

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Background: Papanicolaou (Pap) smear has proved to be one of the most successful methods of cancer detection available. Like most other screening tests, false negative Pap smear does occur and has been most frequently attributed to sampling errors, resulting in absence of abnormal cells. Little has been studied on the impact of intrinsic pathophysiology of cervix itself, such as parakeratosis, in the cause of false negativity. The possible relationship between cervical parakeratosis and negative Pap smear was investigated in this study.

Design: Total 465 cases with diagnostically adequate cervical biopsies and Pap smears performed concurrently at our institution were studied. There were 143 cases with concordant diagnosis of low grade intraepithelial lesion (LSIL; Pap smear with LSIL and biopsy with HPV cytopathic effect or cervical intraepithelial neoplasia (CIN I)), 27 of high grade squamous intraepithelial lesion (HSIL; Pap smear with HSIL or atypical squamous cells-cannot exclude HSIL and biopsy with CIN II or CIN III), and 179 of negative diagnosis for both biopsy and Pap smear. There were 116 cases with discordant diagnosis: 91 cases fell into the group with biopsy of LSIL and negative Pap smear and 25 into the group of negative biopsy and Pap smear of LSIL.

Results: Diagnostic discordance between concurrent biopsy and Pap smear occurred only in the cases of LSIL (biopsy of LSIL and negative Pap smear or Pap smear of LSIL and negative biopsy). No significant discordance between concurrent biopsy and Pap smear was found in the cases with HSIL. Cervical parakeratosis was fairly common, particularly in the group with concurrent biopsy of LSIL and negative Pap smear, in which 94.5% of its biopsies displayed intact parakeratosis overlying the lesions. Additionally, in this group, there was either no ongoing squamous metaplasia present in the transformational zone or lesional cells present only in the base of these ongoing squamous metaplastic epithelia without involvement of the surface. In contrast, in diagnostically concordant cases of squamous intraepithelial lesion for both biopsy and Pap smear, parakeratosis is less frequent (75.3%) and in the cases of parakeratosis present, the surface was frequently superficially eroded or the surface of ongoing squamous metaplasitic epithelium was uninvolved by lesional cells.

Conclusions: Parakeratosis is an important cause for the discordance between concurrent positive biopsy and negative Pap smear in an adequate specimen. Recognition of this phenomenon is of clinical significance for patient management and follow-up.

397 Diagnostic Utility of Mammaglobin and GCDFP-15 in the Identification of Metastatic Breast Carcinoma in Fluid Specimens

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Background: Differentiation of metastatic breast carcinoma from other malignancies in fluid specimens based on morphology alone can be a diagnostic challenge. Immunocytochemistry is often employed in the differential diagnosis. Expression of the mammaglobin gene and protein have been demonstrated in neoplastic breast tissues and normal breast epithelial cells. In this study, we evaluated the efficacy of mammoglobin as a marker for metastatic breast carcinoma in fluid specimens and compared it to GCDFP-15 (Gloss Cystic Disease Fluid Protein-15 or BRST-2), a commonly used marker.

Design: Forty formalin-fixed paraffin-embedded cell blocks from pleural and peritoneal fluid specimens containing metastatic carcinoma were retrieved. They included 15 breast carcinomas and 25 non-breast carcinomas (10 lung adenocarcinomas, 10 ovarian carcinomas, 3 carcinomas of gastrointestinal tract, and 2 urothelial carcinomas). Cell block sections were immunostained with monoclonal antibodies against mammaglobin (clone: 304-1A5, Dako Inc. Carpinteria CA, 1:200) and GCDFP-15 (clone: D6, Signet Inc. Dedham MA, 1:200). Both antibodies required heat-induced antigen retrieval. Positivity stain for both antibodies was defined as the presence of cytoplasmic staining in 10% or more carcinoma cells irrespective of its intensity. Statistical analysis was performed using Fisher's exact test.

Results: Thirteen (87%) and 7 (47%) breast carcinomas showed positive staining with mammaglobin and GCDFP-15, respectively. Three (12%) non-breast carcinomas (2 ovarian and 1 colonic) showed positive mammaglobin staining; 1 (3%) non-breast carcinoma, a lung primary, demonstrated positive GCDFP-15 staining. The difference in mammoglobin and GCDFP-15 staining between breast and non-breast carcinomas was statistically significant ($p<0.05$). The sensitivity of mammaglobin and GCDFP-15 in identifying metastatic breast carcinoma was 87% and 46%, respectively. The specificities for mammaglobin and GCDFP-15 in identifying metastatic breast carcinoma was 88% and 96%, respectively.

Conclusions: Both mammoglobin and GCDFP-15 are specific markers for metastatic breast carcinomas in cell block fluid specimens. However, mammoglobin is a more sensitive marker than GCDFP-15 and therefore a more superior marker when identifying metastatic breast carcinoma.

398 High Sensitivity and Specificity of Reflex HPV Test for Detecting Significant Cervical Lesions in Patients with Cytologic Diagnosis of Atypical Glandular Cells: A 5-Year Cleveland Clinic Experience

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Background: Reflex human papilloma virus (HPV) testing for ASCUS has improved with high sensitivity and specificity in detecting CIN lesions. However, it lacks of guidelines in performing reflex HPV testing on atypical endocervical glandular cells (AEC) before colposcopy. As overall histopathologically-proven abnormal endocervical glandular lesions among patients with cytological diagnosis of AEC is relatively low, We hereby report our past 5-year experience for performing reflex HPV test in patients with AEC diagnosis and assess the potential role of reflex HPV testing in guidance of subsequent colposcopy-directed cervical biopsy/currattage in a large tertiary-care hospital setting.

Design: All AEC cases cytologically diagnosed from July, 2001 to June, 2006 were retrieved from our database. Histopathologic diagnoses and the results of HPV test using the Hybrid Capture 2 (HC-II) method were reviewed. The most severe histopathologic diagnosis was recorded and HPV test results were correlated.

Results: Of total 332,470 Pap tests using ThinPrep method performed in a 5-year period, 387 cases (0.1%) of AEC were detected. Of those, 353 cases (91.2%) had histopathologic follow up. Reflex testing of high risk HPV by HC-II was performed in 317 cases (89.8%). Histopathologic examination of the 64 HPV positive AEC cases revealed 18 cases of endocervical adenocarcinoma in-situ (AIS)/adenocarcinoma, 22 cases of CIN2/3, 14 cases of CIN1, and 10 cases of benign cervix. Among 253 of the HPV negative AEC, AIS/adenocarcinoma was found in only 3 cases, CIN2/3 in 1 case, CIN1 in 2 cases, and 12 cases had endometrial hyperplasia or adenocarcinoma. Cervical AIS/adenocarcinoma was found in 28% of HPV positive AEC patients and only 0.9% of HPV negative patients ($P<0.0001$). When significant glandular and squamous (CIN2/3) lesions combined, 62.5% of the lesions were detected in HPV positive AEC cases compared to 1.6% in HPV negative AEC cases ($p<0.0001$).

Conclusions: Reflex HPV DNA testing has a high sensitivity (40/44, 91%) and high specificity (249/273, 91%) in detection of cervical AIS/adenocarcinoma and high grade squamous dysplasia (CIN2-3) among patients with AEC cytologic diagnosis. It also has a positive predictive value of 62.5% and a negative predictive value of 98%. Our study indicates that reflex HPV testing for cytologic diagnosis of AEC can be a useful ancillary tool in selection of high-risk patients for colposcopy.

399 Root Causes of False Positive (FP) Diagnosis of Papillary Carcinoma (PC) in Fine Needle Aspiration (FNA) of the Thyroid

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Background: FNA has supplanted most other tests for preoperative evaluation of thyroid nodules. FP diagnosis of PC will usually result in unnecessary thyroidectomy. Our objective is to determine the underlying causes of FP diagnosis of PC in thyroid FNA biopsy through a retrospective analysis.

Design: Our files were searched for positive or suspicious diagnosis of PC between 2000 and 2005. Cases with available surgical excision were selected. For the purposes of this study, suspicious diagnosis was included as FP in order to identify the maximum number of reasons for misdiagnosis. FNA slides were evaluated for cellularity, morphologic findings, obscuring blood, stain quality, and air-drying artifact. Surgical excision slides were reviewed for correlation. Patient's age and sex, nodule size and location were obtained.

Results: Our search resulted 303 (227 F, 76 M, mean age 49.4 years) patients with suspicious or positive FNA diagnosis of PC who underwent thyroidectomy. Among those, 13 were FP (12 suspicious, 1 positive). The excision showed 8 nodular goiters (NG), 2 follicular adenomas (FA), and 3 Hashimotos thyroiditis (HT). Among 8 NG cases, 4 had pseudo-papillary hyperplasia; 1 showed cystic degeneration with reactive atypia; 1 was called suspicious due to presence of a fragment of reactive stromal cells, which was evident on excision; 1 had few psammoma bodies in the FNA which were not seen in excision; 1 NG case was probably overcalled as result of air-drying artifact. Notably, none of the NG cases had nuclear inclusions. The two FA cases had papillary hyperplasia without PC features on excision. Among 3 HT cases, 2 were caused by the scarcity of lymphocytes in background. 1 was caused by misinterpretation of cohesive germinal center cells as follicular epithelium (confirmed by CD45 stain retrospectively). All 13 cases had benign clinical course after average 4 years of follow up (range 2-7 years).

Conclusions: 1). The most frequent cause of FP diagnosis of PC is over interpretation of few atypical groups of cells in NG; the excision usually shows pseudo-papillary hyperplasia or cystic degeneration with reactive epithelial cells and stroma. 2). Cohesive germinal center cells in HT can rarely mimic follicular epithelium. 3). The scarcity of lymphocytes and high cellularity of reactive follicular cells in HT can rarely be a pitfall, if we rely heavily on the presence of lymphocytes for the diagnosis of HT. 4). Over interpretation of few psammoma bodies in NG and sub-optimal smears, such as air-drying artifact may cause overdiagnosis.

400 The Diagnostic Value of the Thinprep Pap Test in Endometrial Carcinoma: A Prospective Study with Histological Follow-Up

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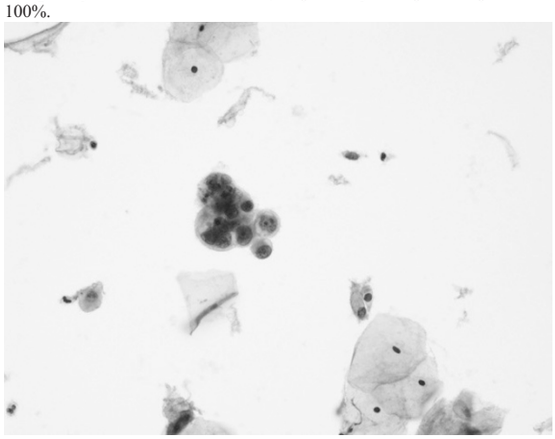
Background: Studies demonstrate the ThinPrep Pap test may provide accurate detection of endometrial carcinoma. The purpose of this study is to prospectively examine the diagnostic potential of ThinPrep Pap test in the detection of endometrial carcinoma.

Design: ThinPrep Pap test slides were collected from 3 patient groups: women> 50 years old with atypical glandular cells on pap; endometrial cells in woman $\geq$ 40 years old in otherwise normal pap smears; or women of any age with adenocarcinoma on Pap test. Pap-stained slides were independently reviewed by one of the investigators. Each case was assigned to one of 4 diagnostic categories: 1) within normal limit (WNL); 2) atypical glandular cells (AGC); 3) atypical endometrial cells (AEC); or 4) adenocarcinoma, probably endometrial origin. Subsequent patient follow-up diagnosis was obtained for the cytological-histological correlation.

Results: Of 106 patients identified, 60 had histological follow-up. For all eight cases interpreted by cytology as positive (Figure 1), endometrial carcinoma was confirmed histologically. Among 25 patients with normal endometrial cells present, histological follow-up showed benign endometrium. 82.4% (14/17) of cases classified as AEC had benign histological follow-up; the remaining 3 cases proved to have endometrial carcinoma. All 11 cases classified as AGC had benign histological follow-up in which endometrial polyps or other benign conditions were responsible for the cytological findings. When AEC and AGC are considered as negative cytological parameters

for endometrial carcinoma, the sensitivity and specificity of detecting endometrial malignancy were 72.7% and 100%, respectively. The positive predictive value is 100%.



Conclusions: In this prospective study, we demonstrate that Thin Prep Pap test has a reasonably high sensitivity and/or specificity in detecting endometrial carcinoma. Based on our findings, the ThinPrep Pap test may be used as a primary screening test for endometrial carcinoma for high risk population.

401 Urothelial Cell Clusters in Voided Urine Cytology Specimens: A Histologic and Cytologic Review of 146 Patients

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Background: The evaluation and classification of atypical urine cytology specimens remains challenging and controversial. The presence of urothelial cell clusters in voided urine specimens has historically been considered an atypical finding, suggesting an increased risk of urothelial neoplasia. However, recent literature has not only questioned the value of reporting urothelial cell clusters, but suggested it is misleading. The aim of our study was to determine the significance of urothelial cell clusters in voided urine cytology specimens at our institution.

Design: The final reports of 3135 consecutive voided urine specimens over a 7 year period (2000-2006) were searched for cases interpreted as atypical based only on the presence of urothelial cell clusters without other significant cytologic atypia. Correlation with the results of subsequent histologic (within 60 days) and cytologic specimens was performed.

Results: 167 specimens (5.33% of all voided urine specimens) from 146 patients (male, 99; female, 47; mean age, 58.9 years) were identified with 15 patients having multiple specimens. Histologic and cytologic follow-up was available in 26 (17.8% of 146 patients) and 59 (40.4%) patients, respectively. 61 (41.8%) patients had no further specimens at our institution. In patients with histologic follow-up, 14 patients (9.6%) had low-grade urothelial carcinoma (UC), 2 (1.4%) had high grade UC, and 1 (0.7%) had urothelial dysplasia. In patients with cytologic follow-up, 1 (0.7%) patient had high grade UC, 6 (4.1%) had atypical findings, and 11 (7.5%) had urothelial cell clusters in subsequent voided specimens. Additionally, two patients underwent subsequent nephrectomy for renal neoplasms (papillary renal carcinoma and cystic nephroma). The remaining patients with follow-up (49, 33.6%) had benign/reactive findings. In total, 17 patients (11.6%) had a subsequent diagnosis of urothelial carcinoma. Of those 17 patients, 9 (52.9%) had no prior history of urologic malignancy at our institution.

Conclusions: The presence of urothelial cell clusters in voided urine specimens correlates with a measureable risk of underlying urothelial neoplasia. Their presence in voided urine specimens should continue to be reported to clinicians to facilitate appropriate clinical follow-up.

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402 Immonuhistologic Determinants of Aggressiveness in Basal Cell Carcinoma

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Background: Basal cell carcinoma (BCC) is a very common malignant skin tumor that rarely metastasizes but is often locally aggressive. Search for clinically useful markers of aggressiveness is still on-going. Bcl2, β-Catenin, Cyclin D1, hMSH2 and αSMA are previously reported but inconlusively, to have potential for predicting BCC aggressiveness. We therefore studied their expression as prognostic indices, in a group of clinically proven aggressive BCC from the head and neck region.

Design: The study materials consisted of 28 cases of aggressive BCC based on >2 recurrences, tumor size >3 cm, and infiltration of deeper structures. 35 randomly selected cases of BCC were also studied as controls. Representative tissue sections were immunostained and the expression of the gene products of interest was evaluated using a semiquatitative scale of 0-4. Also, their topographic locations were noted. The expression of each gene product was compared between the tumor cells and the corresponding non tumor cells in the same tissue section. The results of the two study groups were subjected to statistical analysis and the gene products with statistically significant dysregulation were analyzed further (by logistic regression) to generate reliable immunohistologic criteria for determining the aggressiveness of BCC. The accuracy of the criteria was tested using the Omnibus tests of model coefficients.

Results: As a marker of tumor aggressiveness, upregulation of Bcl2 in the tumor cells was marginally significant ($p=0.047$), upregulation of hMSH2 was significant in both tumor cells and the non tumor internal control cells ($p=0.026$ and 0.003 respectively); expression of αSMA was restricted to the stroma in aggressive tumors ($p<0.001$) and to the tumor cells in the non aggressive tumors ($p=0.029$). Stromal expression of αSMA was highy predictive of aggressive behavior (p,0.001; accuracy 87%). Logistic regression combining the expression of αSMA and hMSH2 yielded a predictive model with 97% accuracy ($p<0.001$). Neither the expression of β -catenin nor that of Cyclin D1 was statistically significant for predicting aggressiveness of BCC.

Conclusions: Aggressive BCC express αSMA in the stroma while non aggressive BCC express αSMA in the tumor cells. Stromal expression of αSMA is an accurate, reliable, and easy to use marker of aggressiveness in BCC and can be used in clinical practice for surgical therapeutic decisions.

403 D2-40 a Novel Immunohistochemical Stain as a Complementory or Possible Replacement to Factor XIIIa and CD34 Immunoperoxidase Stains in Differentiating Dermatofibroma and Dermatofibrosarcoma Protuberance

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Background: Usually differentiating between dermatofibroma (DF), particularly cellular variant and dermatofibrosarcoma protuberance (DFSP) in excisional biopsies are straightforward. However, differentiating between these two entities may become problematic in superficial biopsies. Both DF and DFSP are composed of dermal spindle cell proliferation, which may also demonstrate storiform pattern. Although changes of overlying epidermis as well as grenz zone are soft signs of dermatofibroma, in a minority of cases the distinction is problematic. Majority of dermatofibromas and DFSPs are positive for factor XIIIa and CD34 immunostains, respectively. However, reportedly there are some cases revealing overlapping staining and conflicting results. In this study we compared the results of different immunoperoxidase stains.

Design: 20 cases of DF (including 6 cellular variant) and 24 cases of DFSP were selected from archives of Department of Anatomic Pathology at Sunnybrook Health Sciences Centre, University of Toronto. We applied Factor XIIIa, CD34 and monoclonal mouse anti D2-40 immunoperoxidase stains to formalin-fixed, paraffin-embedded tissue sections. D2-40 immunoperoxidase stain identifies a 40 kD O-link sialoglycoprotein present on a variety of tissues including testicular germ cell tumors as well as lymphatic endothelium.

Results: All 20 (100%) cases of DF revealed strong and diffuse D2-40 staining of spindle cells as well as stroma. Factor XIIIa showed strong and diffuse positivity in spindle cells, more prominent at the periphery of lesions. All spindle cells were negative for CD34 immunostain except one case revealing focal CD34 positivity. Interestingly this focus was negative for D2-40 (mirror image). All DFSPs revealed strong and diffuse positivity for CD34 and negativity for factor XIIIa. 4/24 (16%) cases of DFSPs showed positivity by D2-40, however the staining pattern was faint, patchy and non-crisp, in contrary to diffuse, strong, granular and sharp staining in DF.

Conclusions: D2-40 appears to be a novel immunohistochemical stain, which is 100% positive in dermatofibromas, including cellular variant. It may show focal and faint stromal staining in DFSP. We suggest D2-40 utilization is complementary or even replacement to factor XIIIa and CD34 immunostaining in superficial biopsies in which the junction of dermis and subcutaneous tissue is not present for definitive diagnosis.

404 Characterization of Epidermal Merkel Cell Density Adjacent to Merkel Cell Carcinomas

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Background: Merkel cell carcinoma (MCC) is a unique and highly aggressive neuroendocrine carcinoma of the skin. It has an incidence of 0.23 per 100,000 people in the United States. Although relatively rare, the grave prognosis makes it an area of particular clinical concern. The pathobiology of MCC is poorly understood. It is well known that MCC is typically an intradermal tumor, but there very rare reports of MCC in-situ. Whether or not there is a pre-neoplastic state that acts as the precursor lesion to the invasive tumor is not known. The aim of this study was to discern whether or not there is Merkel cell hyperplasia within the epidermis overlying the invasive tumor.

Design: A total of 21 resection specimens for MCC with overlying epidermis were identified in the files of Washington University from 1989 to the present. The cases, along with 21 site-matched controls, were immunostained for chromogranin-A (chr-A) and cytokeratin 20 (CK20) on 4-micron thick sections using standard autostainer protocols. Sections were examined by one study pathologist (EB), and the number of Merkel cells quantified in the epidermis and hair follicles overlying the tumor. A total of 5 linear mm of epidermis directly above the tumors as well as the entire epidermis on control sections (2-49 mm) was examined and counted for positive cells in each case.

Results: MCC cases had an average of .49/.50 Merkel cells per mm (for chr-A and CK 20, respectively), whereas normal cases showed .66/.95. The density of Merkel cells within the epidermis adjacent to MCC was significantly less ($p < 0.5$) than that of normal controls (Wilcoxon signed rank test). The number of Merkel cells counted on sections stained with chr-A and ck 20 was correlated, ($r = 0.5561$; CI 0.17-.79) indicating that they are both good markers for Merkel cells. In addition, two types of morphologically distinct Merkel cells, those with dendritic processes and those without, were identified in all specimens. The dendritic-type appeared to be more concentrated around the hair follicles while the non-dendritic type tended to be found in the stratum basale.

Conclusions: The density of Merkel cells in the epidermis overlying MCC is not increased when compared to site matched controls. Unlike melanomas and certain carcinomas, an intraepidermal proliferation of neoplastic cells does not appear to precede the development of MCC in most cases.