326 The Pathology of TRI-Tech Leaflet Escape.

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Background: The TRI-Tech valve is a low-profile mechanical bileaflet valve prosthesis with a rotating pivot system. Leaflet escape due to pivot fracture was reported and the implantation program interrupted worldwide. The fracture was ascribed to pivot height asymmetry.

Design: To assess whether the asymmetry was an isolated defect or intrinsic to other commercialized TRI-Tech valves, 3 TRI-Tech valves with leaflet dislodgement implanted in 3 patients (pts) two in aortic and one in mitral position and 150 unimplanted TRI-Tech valves, 19-31 mm in size, were studied. Both the pts with aortic escape died suddenly at 10 days and 39 months after surgery and the escaped emidisc was found in the thoracic aorta and in the left common iliac artery, respectively. The pt with mitral leaflet escape had a cardiogenic shock at 22 months, was successfully reoperated and the escaped leaflet was found in the left common iliac artery. Tab height and asymmetry measurement (Δ between tab heights from the leaflet base) was performed in all cases.

Results: In all the escaped discs one pivot disappeared, fractured at the base, thus explaining leaflet dislodgment from the hinge and its distal escape. Small thrombus deposition was observed within the hinge of the fractured tab in the aortic patient who died suddenly at 40 months from reoperation.The asymmetry was over 0.35 mm in all (0.55 and 0.40 mm in the aortic, and 0.46 in the mitral prostheses), and the fracture involved the lower tab in 2 and the higher tab in one. The asymmetry was observed in all unimplanted valves was less than 0.08 mm in 69 (46%), in between 0.08 and 0.20 in 66 (44%), 0.20 to 0.35 mm in 14 cases (9%), and over 0.35 mm in one case. Only in one out of 150 unimplanted valves the asymmetry was as high as observed in the fractured clinical valves.

Conclusions: Asymmetry was present in all unimplanted valves, however, in only one device it was of such size as observed in failing devices. This means that pivot fracture occurred when the height asymmetry was particularly severe. The more the asymmetry the early the fracture occurrence. Pivot rupture with leaflet escape was rarely reported in other valve models. The TRI-Tech quality control did not foresee pivot symmetry check. Since tolerance for pivot asymmetry is unknown and risk of rupture unpredictable, interruption of implant program with prophylactic replacement of implanted TRI-Tech valve was judicious.

327 Chromatin Remodeling and Cell Cycle Activity in Periinfarction Myocardium.

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Background: Proliferative activity of cardiac myocytes has been observed in periinfarction myocardium. To access DNA template, chromatin remodeling and DNA unwinding is required. Enhancer of zeste homolog 2 (EZH2), a key component of polycomb repressive complex 2, regulates cell proliferation by histone H3 methylation at lysine 27. Brahma-related gene 1 (Brg1) is the ATPase subunit of a large chromatin remodeling complex. The aim of this study was to determine if EZH2 and Brg1 expressed in periinfarction myocardium.

Design: Ten cases of left ventriculectomy for the placement of left ventricle (LV) assistance device in patients with acute myocardial infarction less than 2 week old and 2 cases of normal hearts unsuitable for implantation were studied. Four micron sections of LV were stained with antibodies against EZH2 (Leica, clone 6A10, 1:100), Brg-1 (Santa Cruz, clone G-7, 1:100), and Ki-67 (DAKO, clone MIB1, 1:100) using a kit (EnVison™ Flex+ Dako) and an automated immunostainer.

Results: Rare non-cardiac myocytes in normal hearts and remote areas of infarcted hearts showed nuclear positivity for EZH2 and Ki-67, but no staining for these markers was detected in cardiac myocytes in the same areas. In periinfarction myocardium, there was significant increase in Ki-67 and Brg1 positive non-cardiac myocytes. Strong nuclear staining for these markers was also detected in 5 to 10% cardiac myocytes in periinfarction zones. More importantly, serial section staining revealed that there was co-expression of these markers in cardiac myocytes of periinfarcion myocardium. Brg-1 was weakly expressed in both cardiac myocyte and non-cardiac myocytes in normal hearts and remote areas of infarcted hearts. There was significant increase in Brg-1 expression in periinfarcion myocardium.

Conclusions: Cell cycle entry in cardiac myocytes of periinfarction myocardium is accompanied by increased expression of EZH2 and Brg1. This result suggests there is chromatin remodeling and histone modification in cardiac myocytes of periinfarction myocardium.

327A Novel PKP2 Mutations in Sudden Death Due to Arrhythmogenic Right Ventricular Cardiomyopathy and Sudden Unexpected Death with Normal Cardiac Morphology.

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Background: Sudden deaths in adults are often of cardiac origin and can be attributed to Arrhythmogenic right ventricular cardiomyopathy (ARVC) and sudden adult death syndrome (SADS). ARVC has been linked to mutations in Plakophilin2 (PKP2), which is a desmosome related protein with numerous armadillo repeats. SADS accounts for up to 30% of sudden death in adults, and a subset of cases has also been linked to ion channel mutations, but not to PKP2 thus far. We determined mutational status of PKP2 in a series of patients dying suddenly with ARVC and SADS.

Design: 33 cases of sudden unexpected death of cardiac etiology determined by full forensic autopsy were studied. 7 cases were witnessed sudden deaths in patients with normal hearts; 4 men (aged 32 ± 11) and 3 women (aged 24 ± 16). 26 cases had typical morphologic features of ARVC (fibrofatty infiltrates in the right ventricle and subepicardium of the left ventricle); 19 men (aged 35 ± 17 years) and 7 women (aged 33 ± 16 years). We sequenced all 14 exons of PKP2 in DNA extracted from post-mortem tissues of the 26 patients dying with ARVC and 7 with SADS. The primers used in this study were designed using the Primer Express 3.0 software. Direct sequencing for both sense and antisense strands was performed with a BigDye Terminator DNA sequencing kit on a 3130 Genetic Analyzer with SeqScape software.

Results: PKP2 mutations were identified in 7 of 26 DNA samples from patients with ARVC. Of the 7 mutations, 3 were likely significant, and two of which (L64PfsX22 and N642del) are novel mutations that has not been reported in patients with ARVC. PKP2 mutations were also identified in 3 of 7 cases of SADS, and one novel mutation (F339S) is likely significant.

Conclusions: PKP2 mutations are not specific for ARVC and may also be found in patients with sudden death without morphologic findings (SADS). Three new mutations of PKP2 are described (F339S,N642del,L64PfsX22) in patients with ARVC and SADS.

328 Oxidative Stress and ERK1/2 MAP Kinase Mediate Cardiomyocyte Injury in Transthyretin Cardiac Amyloidosis.

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Background: Transthyretin (TTR) is associated with two forms of cardiac amyloidosis: familial (mutant TTR) and systemic senile amyloidosis (wildtype TTR). Approximately 4% of African Americans are heterozygous for V122I variant TTR, a mutation associated with familial amyloidotic cardiomyopathy. The mechanisms of TTR-induced cardiac injury remain elusive. Markers of oxidative stress have been associated with TTR amyloid deposits in peripheral nerve and we previously reported that oxidative stress and ERK1/2 activation mediate cell death in lung epithelium. We investigated the potential role of oxidative stress and ERK1/2 activation in mediating TTR-induced cardiomyocyte injury.

Design: Cases of TTR cardiac amyloidosis and age-matched controls were identified from 2007-2010 autopsy records. TUNEL and 8-OH-dG staining was performed on formalin-fixed paraffin-embedded sections from left ventricle. The TTR gene was sequenced using genomic DNA extracted from the paraffin blocks. Cultured rat cardiomyocytes were exposed to TTR fibrils formed by incubating wildtype TTR under acidic conditions. Apoptosis was assessed by TUNEL staining and Annexin V flow cytometry. Oxidative stress was examined by Western blot for heme oxygenase-1 (HO-1), reactive oxygen species (ROS) production, and 8-OH-dG staining. ERK1/2 activation was measured by Western blot of phospho-ERK1/2.

Results: Four cases of TTR cardiac amyloidosis (average age 82.8 years; range 80 to 87 years) were identified. All patients had presented with chronic heart failure and arrhythmia. TTR gene sequencing identified mutant TTR (V122I) in 3 cases and wildtype TTR in 1 case of cardiac amyloidosis. Positive staining of TUNEL and 8-OH-dG was more prominent in cases of cardiac amyloidosis than in control heart tissue. Rat cardiomyocytes treated with TTR fibrils showed more ROS production, HO-1 expression, phospho-ERK1/2, and apoptosis than untreated cardiomyocytes. Inhibition of ERK1/2 activation by PD98059 or ROS production by diphenylene iodonium ameliorated TTR fibril-induced oxidative injury and apoptosis.

Conclusions: Apoptosis and oxidative injury are increased in hearts of patients with cardiac amyloidosis compared with age-matched controls. Oxidative stress, ERK1/2 activation, and apoptosis are involved in TTR-induced injury of cultured rat cardiomyocytes. Inhibition of oxidative stress and ERK1/2 activation may provide a potential mechanism for prevention and treatment of cardiac injury associated with TTR cardiac amyloidosis.

Cytopathology

329 Heterogeneity of the Testis, an Important Clinical Fact for Infertile Azoospermic Men Undergoing Fine Needle Aspiration of the Testis.

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Background: Azoospermic infertile male patients have to resort to testicular fine needle aspiration to search for sperms. These sperms can be used for intracytoplasmic sperm injection into the partner's ova in the assisted reproduction procedure. Finding sperms in the testis is of paramount importance for such couples.

Design: 1620 azoospermic patients had undergone testicular fine needle aspiration for the assessment of their spermatogenesis status. Each patient has been sampled from 10 specific locations, 5 from the right testis and 5 from the left testis, using 27 gauge needles. Patients are classified according to the presence or absences of sperms and its quantity into the following stages: I) Sertoli cells only, II) Spermatogenesis arrest at early stage, III) Spermatogenesis arrest at late stage, IV) sperm heads only V) Hypospermatogenesis (sperms were seen) and VI) Obstructive azoospermia. Hypospermatogenesis is also divided into 4 classes according to the amount of sperms: A) very marked Hypospermatogenesis, B) marked Hypospermatogenesis C) moderate Hypospermatogenesis D) mild Hypospermatogenesis. In each patient, the testis is classified as homogenous or heterogeneous in regard to spermatogenesis status. The testis is considered heterogeneous when 5 testicular sites or less show the cytomorphological changes of the highest stage of spermatogenesis diagnosed in that particular patient.

Results: Patients' ages ranged from 20-55 years with average age 28.5 years. Testicular fine needle aspiration from the 10 sites from each patients showed that the testis was homogenous in 1083 cases (67%) and heterogeneous in 537 cases (33%). The

heterogeneity occurred in stage II-VI as follows: Stage II: form 56 cases (out of 537) (10% of heterogeneous cases); Stage III: 54 cases (10%); Stage IV: 55 cases (10%); Stage V: patients with Hypospermatogenesis form 68.5 % of heterogeneous cases and distributed as follows: Very marked Hypospermatogenesis: 43% of heterogeneous cases; Marked Hypospermatogenesis 14%; Moderate Hypospermatogenesis 9.5%, Mild Hypospermatogenesis 2%. Stage VI: form 1.5% of the heterogeneous cases.
Conclusions: Testis is a heterogeneous organ in regard to sperm production in infertile men in about 1/3 of the cases. The lesser amount of sperms produced, the more likely that the testis will be heterogeneous. It is essential to sample the testis from multiple sites to search for sperm production. This fact is important for sampling the testis in infertile men entering assisted reproduction program.

330 PAX8: A Sensitive Marker To Identify Cancer Cells of Gynecologic Origin in Pelvic Washings.

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Background: Pelvic washings are a routine component of the staging procedures for many gynecologic tract cancers, and the correct interpretation of the pathologic changes in the resultant specimens may have a direct impact on post-surgical patient management. However, inconclusive changes in washing specimens are not infrequently encountered. PAX8 is a nuclear transcription factor that has recently been recognized as a useful biomarker in distinguishing ovarian carcinomas from breast cancers and mesotheliomas. The objective of this study was to assess the diagnostic utility of PAX8 in pelvic washing specimens.
Design: Pelvic washing samples with cell blocks from 53 patients with a variety of neoplastic and non-neoplastic pathologic processes were retrieved. These included 15 "positive", 28 "atypical or suspicious but non-diagnostic", and 10 "negative" cases based on cytology reports. Immunohistochemical studies for PAX8 and Calretinin were performed on all cases. Ovarian cancer, which has a known high level of PAX8 expression, served as positive controls. The final diagnosis for each case was based on the conventional gold standard, which was based on the totality of all clinicopathologic findings.
Results: All "positive" cases were PAX8 + and Calretinin -. All "negative" cases were PAX8 -. The group with inconclusive diagnoses showed the following distribution of findings: 14 PAX8 +, 13 PAX8 – and 1 non-contributory. The 14 positive cases included ovarian high-grade serous carcinoma (7), ovarian low-grade serous carcinoma (2), serous borderline tumor (2), Sertoli-Leydig cell tumor (1), endometriosis (1), and tubal torsion (1). The 13 negative cases included endometrial endometrioid carcinoma (5), cervical adenocarcinoma (2), metastatic colon cancer (2), metastatic breast cancer (1), mesothelioma (1), uterine adenomyosis (1), endometrioma (2). The false positive and false negative rates for PAX8 alone were 21% and 0% respectively. However, when combining with Calretinin results and clinicopathological findings, the sensitivity and specificity of PAX8 for diagnosing cancer cells were both over 95%.
Conclusions: PAX8 is a sensitive and specific biomarker to detect cancer cells in pelvic washing specimen when it is combined with Calretinin staining. The most common PAX8 false positive cells are benign cells of Mullerian origin. Clinicopathological correlation is necessary in this setting.

331 BRAF Mutation in Thyroid FNA Specimens Enhances Predictability of Malignancy in Thyroid Follicular Lesion of Undetermined Significance.

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Background: The Bethesda 2007 Thyroid cytology classification defines follicular lesion of undetermined significance (FLUS) as a heterogeneous category of cases that are not convincingly benign nor sufficiently atypical for a diagnosis of follicular neoplasm or suspicious for malignancy. In our institution, we refer to these cases as "indeterminate" and they are further subclassified into two: 1) Low cellularity with predominant microfollicular architecture and absence of colloid (INa) and, 2) Nuclear features not characteristic of benign lesions (nuclear atypia) (INb). BRAF mutation occurs in 40-60% of papillary thyroid carcinoma (PTC). Such mutations are associated with a more aggressive phenotype of PTC. In this study, we examined and correlated our "indeterminate" cases with the result of BRAF mutation analysis and surgical pathology outcome.
Design: Thyroid FNA cytology specimens with an "indeterminate" diagnosis and a concurrent BRAF V600E mutation analysis were selected from our files. BRAF mutation analysis was performed by PCR combined with single strand conformation polymorphism gel electrophoresis using the remnant of samples collected for thin-layer processing in each case. Surgical pathology reports were reviewed for the final outcomes in these patients.
Results: Of the 49 indeterminate cases with BRAF mutation analysis, only 25 (51%) had follow-up with surgical intervention (24% of INa and 56% of INb). Ten cases (1 INa and 9 INb) had BRAF V600E mutation, 14 had no mutation while there was insufficient material in 1 case. All the 9 BRAF positive INb cases had a final diagnosis of PTC. In the 9 BRAF negative INb cases, 4 had a final diagnosis of PTC while 5 were benign lesions. The only BRAF positive INa case had a benign diagnosis. Of the 5 BRAF negative INa cases, 4 were benign while one was a follicular variant of PTC.
Conclusions: The sensitivity and specificity of BRAF mutation in detecting PTC in INb FNA specimens were 69% and 100% respectively, while the positive predictive value was 100%. In the INa category, the sensitivity was very low while the specificity and negative predictive value were both 80%. Our limited data supports the stratification of FLUS into two distinct groups and justifies the use of BRAF mutation analysis to predict the risk of malignancy in the two groups. However, a large number of cases may be needed for a more definitive conclusion.

332 Triage of ASC Cytology Using Biomarkers: A Comparative Study of SurePath and ThinPrep Platforms.

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Background: The objective of this study was to assess and compare the predictability of novel biomarkers, MCM/Top2A (ProEx C, BD), HPV E6E7 mRNA (Proofer, NorChip) and HPV DNA (HC II, Qiagen) in women with ASC (atypical squamous cell) cytology with biopsy confirmed high grade dysplasia or worse (CIN [cervical intraepithelial neoplasia] 2 +) in ThinPrep (Hologic) and SurePath (BD) liquid based cytology (LBC) platforms.
Design: Study population consisted of patients referred for colposcopy in 5 of the 10 Canadian provinces as part of the TPAPT (Transient Persistent And Persistent Transforming) study. Two separate cytology samples from each patient were collected (n=1821); the first sample was collected in ThinPrep PreservCyt medium with the second collected in SurePath medium. PreservCyt specimens were tested for HPV DNA and E6/E7 mRNA while those collected in SurePath tested for MCM/Top2A. Histology confirmed CIN2+ served as the disease end point. Binary logistic regression models were performed to examine the usefulness of biomarker profiles for each of the LBC platforms.
Results: Of 1821 patients, 392 (21.5%) and 251 (13.8%) patients were identified having ASC cytology by ThinPrep and SurePath platforms, respectively. The frequency of CIN 2+ in ThinPrep and SurePath cohorts were 20.9% (82/392) and 22.3% (56/251). Table 1 presents findings of logistic regression analysis for the two groups. No statistically significant difference was found between the odds ratio in each model between the two platforms.

Table 1. Detection of CIN 2+ in ASC cytology based on ThinPrep and SurePath LBC platforms

	ThinPrep (n=392)		SurePath (n=251)	
	Odds Ratio	95% CI	Odds Ratio	95% CI
HC II DNA and E6E7 mRNA	7.3	4.3-12.4	4.6	2.5-8.7
HC II DNA and MCM/Top2A	6.6	3.9-11.3	2.5	1.3-4.6
E6E7 mRNA and MCM/Top2A	7.0	4.0-12.5	4.6	2.1-9.7
HC II DNA, E6E7 mRNA and MCM/Top2A	7.6	4.2-13.5	4.5	2.1-9.7

Conclusions: Our findings show that combining biomarkers are useful in identifying high grade dysplasia in patients with ASC cytology. Although no statistically significant difference was found between the odds ratio in each model between the two LBC platforms, the ThinPrep group showed a stronger predictability than the SurePath cohort by measure of the higher odds ratios.

333 Pattern of Pap Smears in Saudi Arabia.

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Background: Cervical cancer is an important cause of female mortality and morbidity world wide.Large number of studies emphasize that it is a preventable cancer as it is caused by Human Papiloma virus infection(HPV)and the premalignant lesions(PML) associated with this infection can be detected by Pap smear(PS).Cervical Cancer Screening Program(CCSP) is the best way for early detection of the PML.In Saudi Arabia(SA) in which there is no CCSP but there is marked increase in the incidence of abnormal cervical cytology in the last decade.This study evaluated the PS diagnosis in King Adulaiz University Hospital(KAUH) by using the Bethesda system(BS) and compare the result to a similar study from the same institution.
Design: A retrospective study was designed to review all PS from the Cytopathology department of KAUH from January 2005-December 2009.The revised BS diagnostic categories was used for the diagnosis of the PS
Results: Of the 7297 cases reviewed,there were 1254 cases(17.18%)with epithelial cell abnormalities of the total PS examined.The categories include,Atypical Squamous Cell Of Undetermined Significance(ASC-US)were seen in 674 smears(9.23%).Atypical Squamous Cell, cannot exclude High Squamous Intraepithelial Lesion were seen in 60 smears(0.82%).Low grade Squamous Intraepithelial Lesion was seen in 198 smears(2.71%).High Grade Squamous Cell Lesion was seen in 63 smears(0.86%) with mean age incidence(MAI)of 40,42,47, and 45 years respectively.Squamous Cell Carcinoma was seen in 4 smears(0.05)with mean age incidence of 46 years. In the atypical glandular cell category, the Atypical Glandular Cell Not Other Wise Specified there were 232 smears(3.17%).Seven smears(0.09%)of atypical glandular cells favoring neoplasm and 6 smears(0.08%)atypical endometrial cells favoring neoplasm with MAI of 49 and 50 years respectively.
Conclusions: The incidence of abnormal PS was increased 17.8% in comparison to the previous study done in the same institutions 4.7%.ASC-US among total Pap smears examined was significantly increased thereby pushing the number of abnormal Pap smears to a higher side.These changes could be partly to the amalgamation of ASCUS favoring reactive and those favoring neoplastic into one category within the revised BS.We recommend the initiation of HPV testing routinely for all cases with atypical PS with proper treatment and follow up in KAUH as the Bethesda recommendation.A cross-sectional study is required to evaluate the magnitude of abnormal Pap smears in the Western Region of SA with HPV sub typing before subsequent CCSP initiation.

334 Digitally Assisted Review of PAP Smear Cellblock Preparations "Telepapology" Is a Valid Screening/Diagnostic Method.

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Background: The Pap test is a useful method for the detection of precancerous and cancerous lesions of the cervix. To date, the impact of digital imaging on routine day to day cytology remains far from perfect. Cellblock (CB) preparations from discarded/residual conventional and liquid based GYN samples have been shown to be of

diagnostic value. In a pilot study, we have demonstrated the feasibility of utilizing imaging technology to overcome current limitations by digitizing cytologic specimens from CB preparations. Our goal is to develop a web-based diagnostic process in which virtual slides of CBs (TelePAP) could be analyzed remotely, as a model for widespread adoption. Our objective is to compare test performance characteristics of ThinPrep (TP) and TelePAP samples.

Design: The Cellient system from Hologic (Marlborough, MA) was used to prepare CBs. 335 H&E stained CB slides prepared from residual TP samples, including 233 normal, 44 ASCUS, 44 LSIL and 14 HSIL cases, were analyzed. TelePAP slides were obtained using the Aperio digital imaging system (Vista, CA). They were reviewed by 3 cytopathologists and 2 cytotechnologists. Test performance characteristics of TP and TelePAP samples were compared for sensitivity and specificity.

Results: CBs contained optimal amount of material from all cases for meaningful evaluation. The average sensitivity and specificity of the 5 reviewers for the TelePAP method was 81.6% and 87.8%, respectively, for negative cases, 55.1% and 87% for ASCUS cases, 52.0% and 92% for LSIL cases and 49.4% and 98.8% for HSIL cases. Agreement between TelePAP reviewers and TP diagnosis was as follows: Kappa 0.49, 0.61, 0.75, 0.76 and 0.81. Agreement between the 5 TelePAP reviewers for each diagnosis was kappa 0.66. Compared to TP diagnosis, a significant number of ASCUS cases were reclassified as either normal or LSIL using TelePAP method and fewer numbers of LSIL cases were either up or down graded. Time required for digitally scanning CB slides varied from 2-3 minutes. Average time spent reviewing slides was similar to conventional method (2-5 min).

Conclusions: TelePAPology is a feasible method for widespread adoption to achieve high quality specimen preparations. It is as sensitive as TP method and appears to be highly specific for detection of LSIL and HSIL lesions. This method has the potential to revolutionize screening for cervical cancer. It is suitable for routine cytology, in situ and immunohistochemistry testing for HPV and other prognostic markers.

335 Distribution of the Bethesda System-Thyroid Diagnostic Categories in the African-American Population in Conjunction with Surgical Pathology Follow-Up.

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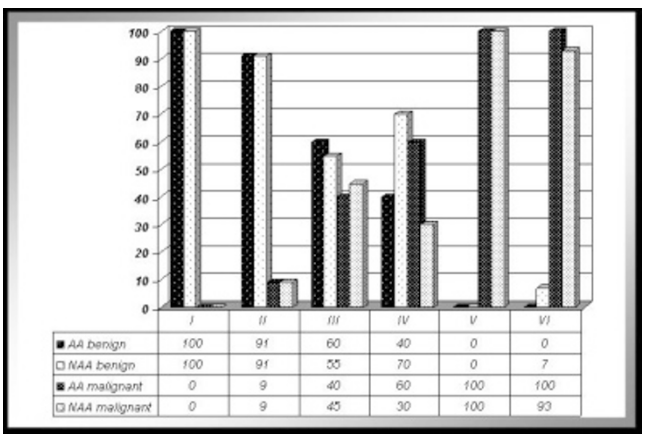
Background: African Americans (AA) have a higher incidence of malignancy compared to Caucasians in the US. However, a recent report indicates that the incidence of thyroid malignancy in AA is half that of Caucasians. The aim of this study is to assess the distribution of malignant *versus* benign thyroid disease in AA in an urban based academic hospital setting. Our study looks at the AA population compared to other racial groups with respect to FNA of thyroid lesions in correlation with final surgical pathology.

Design: We retrospectively reviewed thyroid FNA cytology between January 2005 and August 2010. Consecutive FNA specimens with corresponding follow-up surgical pathology were included. The patients were categorized as (AA) and Non African Americans (NAA) which included Caucasians (C), Hispanics (H), and "Others" (O). The FNA results were classified using the latest edition of the Bethesda System for Reporting Thyroid Cytopathology (TBS-Thy) and the follow-up surgical pathology were used for final categorization.

Results: 238 patients met our inclusion criteria- 139 AA (58%), 99 NAA [36 C (15%), 3 H (1%), 63 O (26%)]. The average age for AA was 51 (range 20-88) and for NAA was 53 (range 25-86) years. There were more females than males in the AA versus the NAA group (83% *versus* 74%). The incidence of thyroid lesions in FNA specimens was similar between these two populations (Table 1). The distribution of benign *versus* malignant diagnoses on follow-up surgical pathology were correlated with TBS-Thy class as summarized in Figure 1.

Table 1

Race	Age (Mean)	I	II	III	IV	V	VI
AA	53	7%	57%	19%	2%	2%	13%
NAA	49	7%	50%	18%	13%	<1%	12%



Conclusions: Our data suggest that distribution of benign *versus* malignant lesion in thyroid FNA with surgical pathology follow-up is similar for TBS-Thy classes I-III, V, and VI in AA *versus* NAA. There was a difference noted in class IV between the two groups, but the small sample size precludes addressing the difference noted.

336 Comparison of Cytologic Features of Gangliogliomas on Crush Preparations.

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Background: Gangliogliomas (GG) are well-differentiated, slowly growing neuroepithelial tumors composed of neoplastic, mature ganglion cells in combination with neoplastic glial cells. The histopathologic features of GG have been well described. However, the cytopathologic features have been sporadically reported in the literature mainly as single case reports. Because of the rarity of this lesion and the confusion with other low-grade astrocytic lesions on cytologic material we undertook a retrospectic study to investigate the cytologic features of this tumor on crush preparations.

Design: A retrospective search of the archives from our institution for a fourteen year period of time revealed a total of 15 cases of GG, all of which underwent frozen section examination with concomitant crush preparations, however cytologic material was available for eleven patients for review, which forms the basis of this study. The clinical and cytologic findings were reviewed.

Results: There were eleven males and four females (M:F, 3:1), ranging in age from 7 to 48 years with a mean age of 25.3 years. The anatomic locations were; temporal lobe (9), amygdala (2), cerebellum (1), hippocampus (1), parietal lobe (1) and frontal lobe (1). The most common clinical presentation was an incidental mass and intractable seizures. The initial frozen section interpretations were; low-grade glioma (4), GG (3), reactive astrocytosis (3), pleomorphic xanthomatous astrocytoma (3) and pilocytic astrocytoma (1), while one case was interpreted as negative. The majority of the tumors show hypercellular smears, predominantly fibrillary background, fine complex capillary background and scattered neuronal cells with eccentrically located nuclei with fine chromatin pattern, prominent nucleoli and indistinct cytoplasmic borders. Non-consistent cytologic features included microcalcifications, intranuclear cytoplasmic inclusions, eosinophillic bodies, binucleation and pilocytic appearance mimicking a pilocytic astrocytoma. Immunostaining was done on paraffin material in all cases using GFAP, Neu-N and synaptophysin and displayed expected results. Clinical outcome was available in all cases and patients are all alive without disease.

Conclusions: The intraoperative cytologic diagnosis of GG is possible. However, due to its rarity and highly variegated cytomorphologic appearance, it could often lead to a more generic diagnosis of "low-grade glioma, NOS". The presence of dispersed neuronal cells with eccentrically located nuclei should raise the possibility of a GG. The main differential diagnosis includes pilocytic astrocytoma and other low-grade gliomas.

337 Evaluation of a Triple Combination of CK20, p53 and CD44 for Improving Detection of Urothelial Carcinoma in Urine Cytology Specimens.

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Background: Expression of CK20, CD44, and p53 have been shown to be useful in differentiating high grade urothelial lesions (CK20+, p53+) from low grade lesions including reactive atypia (CD44+) in surgical specimens. Most urine cytology specimens diagnosed as "atypical" trigger cystoscopy with additional sampling or testing for molecular abnormalities with the Urovision FISH test, both of which increase cost and patient discomfort. Cytology material is generally limited and restricts the use of multiple immunostains. This study was designed to determine the utility of a cocktail of the three stains, CK20, p53, and CD44 (TSC), in urine cytology samples for improving the detection of urothelial carcinoma.

Design: 73 urine cytology specimens (22 negative, 42 atypical, and 9 positive cases) with adequate cellular material on cell blocks were retrieved from the department files between 2007-2010. Smears were screened to confirm adequacy and cytologic diagnosis. 51/73 (69.9%) cases had surgical biopsy and/or FISH testing concurrently or within 6 months of diagnosis. IHC staining with the TSC cocktail was obtained on the cell block for all 73 cases. Positive staining was visualized as red cytoplasmic (CK20), brown membranous (CD44), and brown nuclear (p53). TSC was recorded as positive when CK20 and/or p53 staining was seen in morphologically atypical urothelial cells and as negative if no staining was observed or only CD44 staining was seen.

Results: A positive TSC stain had a sensitivity of 80%, specificity of 92.3%, PPV of 90.9% and NPV of 82.8% for the detection of urothelial carcinoma.

Results of TSC staining in urine cytology specimens

	Cytologic diagnosis (n=73)		
	Negative (n=22)	atypical (n=42)	Positive (n=9)
TSC +	0	18	8
TSC -	22	24	1*

*Low grade papillary urothelial carcinoma (LGPUC) on biopsy

Result of TSC staining correlated with follow-up diagnosis (n=51)

	Negative cytology (n=15)		Atypical cytology (n=28)		Positive cytology (n=8)	
	Negative follow-up	Positive follow-up	Negative follow-up	Positive follow-up	Negative follow-up	Positive follow-up
TSC+	0	0	2	13[1]	0	7
TSC-	15	0	9	4[2]	0	1*

*LGPUC;[1]2 were LGPUC on biopsy; [2]3 were LGPUC and 4th was FISH + only

Conclusions: The triple stain cocktail of CK20, p53 and CD44

1) can be applied to urine cytology specimens with limited material

2) is inexpensive and easy to perform

3) is an effective tool that may be applied for triage of urine samples with an atypical diagnosis

4) has a low sensitivity for identifying low grade urothelial carcinoma

338 The Incidence of Papillary Thyroid Carcinoma (PTC) in Nodules Not Biopsied by Fine Needle Aspiration.

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Background: Fine needle aspiration biopsy (FNAB) of the thyroid is an integral part of the work-up in patients with thyroid nodules. Nodules less than 1.0cm are not usually biopsied unless radiologically suspicious. The aim of our study was to determine the incidence of PTC in thyroid nodules that were not biopsied by FNAB.

Design: A database search, of our Cytology records, identified 249 ultrasound-guided FNAB with surgical follow-up (2006-2010). We grouped the surgical resection specimen into three categories based on the initial FNAB diagnosis (Benign, Suspicious, or Malignant). Radiological and surgical correlation was utilized to determine the incidence of PTC in nodules that were not biopsied by FNAB. These nodules (with PTC) were sorted into three groups based on size (>1.0cm, 0.5-1.0cm, and <0.5cm).

Results:

	Size of Nodules with PTC		
Resection Category	> 1.0cm	0.5-1.0cm	<0.5cm
Benign	5	6	9
Suspicious	1	0	5
Malignant (PTC)	4	10	6
TOTAL	10	16	20

117/249 FNAB had a benign cytologic diagnosis. 20 of these 117 FNAB showed PTC occurring in nodules not biopsied by FNAB [5 nodules >1.0cm, 6 nodules- 0.5 to 1.0cm, and 9 nodules <0.5cm].

23/249 FNAB had a suspicious cytologic diagnosis. 6 of these 23 FNAB showed PTC occurring in nodules not biopsied by FNAB [1 nodule >1.0cm, 0 nodules- 0.5 to 1.0cm, and 5 nodules <0.5cm].

109/249 FNAB had a malignant cytologic diagnosis. 20 of these 109 FNAB showed PTC occurring in nodules not biopsied by FNAB [4 nodules >1.0cm, 10 nodules- 0.5 to 1.0cm, and 6 nodules <0.5cm].

In summary, 46/249 nodules (18% of total) showing PTC were not previously biopsied by FNAB (10 nodules >1.0cm, 16- 0.5 to 1.0cm, and 20 were < 0.5cm).

Conclusions: -Our study shows that a significant number of nodules (18% of total), not biopsied by FNAB, harbor PTC.

-Ten nodules greater than 1.0cm, not biopsied by FNAB, were shown to harbor PTC. Most significantly, 5 of these 10 lesions were in patients with a benign multinodular goiter by ultrasound and FNA.

-This study has significant implications, as radiologic correlation should be an essential part in determining cytologic/histologic correlation.

339 Identification of a DNA Methylation Marker That Differentiates Malignant Mesothelioma from Reactive Mesothelial Cells on Effusion Samples.

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Background: Differentiating reactive mesothelial (RM) proliferations from malignant mesothelioma (MM) in effusions is a challenging diagnostic dilemma for pathologists. Robust, highly sensitive and specific assays are needed to solve this diagnostic problem. Epigenetic changes to DNA, including gene specific promoter methylation and global hypomethylation, are known to occur during tumorigenesis, and there have been several recent studies of MM describing global gene methylation patterns. In this study, we analyzed promoter methylation of several candidate genes for their utility in differentiating RM cells from MM in effusions.

Design: Cell block sections of 31 effusions from patients with MM involvement (17) or with RM proliferations (14) were retrieved from the files of the Cytology Section, Laboratory of Pathology, NCI. Guided by published methylation array data, we selected several candidate genes for detailed promoter methylation analysis. These included several members of the TRAIL/Death Receptor-Decoy Receptor pathway (TRAIL, DcR1, DcR2, Caspase 8), and the retinoic acid responder protein 1 gene, RARRES1. We also assessed Line 1 promoter methylation, as a surrogate for global DNA hypomethylation. Promoter methylation was analyzed using sensitive and quantitative bisulfite pyrosequencing assays.

Results: Of the TRAIL receptor pathway genes, DcR1 displayed the most dramatic methylation differences with MM cases having an average methylation of 27% and the RM cases, 5.8% (p= 0.0009). Using an optimal cutoff for this biomarker as determined by receiver operator characteristic (ROC) curve analysis, this marker had a sensitivity of 88% and a specificity of 100%. While promoter hypermethylation of both DcR2 and TRAIL was also highly sensitive for MM, the specificity of both assays was relatively low, rendering both markers unsuitable for diagnostic use. Neither caspase 8 nor RARRES1 showed significant methylation differences between MM and RM. Only three cases of MM showed significant global hypomethylation as assessed by Line 1 analysis.

Conclusions: Our studies indicate that promoter methylation of Decoy Receptor 1 (DcR1) is a promising marker that differentiates MM cells from RM cells in cytologic effusions. Furthermore, global hypomethylation, using Line 1 as a surrogate marker, was of limited use in distinguishing MM from RM. The identification of DcR1 hypermethylation as a highly sensitive and specific molecular marker for MM has significant implications for the diagnosis of MM.

340 A Comparative Study of the Cytologic and Radiographic Diagnosis of Lesions and Masses of the Kidney, Pancreas, and Liver.

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Background: Radiographic imaging is excellent in detecting a wide variety of intra-abdominal masses and lesions. Fine needle aspiration (FNA) biopsy has been established as a routine method of choice for the pathologic diagnosis of these abnormalities at our institution. Advances in radiographic technology however, are making it possible to correctly predict the pathologic diagnosis by imaging findings alone. The aim of this study was to establish a baseline comparison of the cytologic diagnosis with the radiographic diagnosis of lesions and masses of the kidney, pancreas, and liver.

Design: FNA cases from the kidney, pancreas, and liver were retrieved using our electronic database between January 2007 and December 2009 with exclusion of unsatisfactory specimens and those with no clinical or radiographic follow-up. The cytologic diagnosis for each case was stratified into one of five categories: neoplastic (CN), benign (CB), atypical (CA), suspicious for neoplasm (CSN), and purely descriptive (CD). The radiology report(s) prior to or at time of FNA biopsy from each case was reviewed and stratified into one of six categories: definitive for neoplasm (RN), definitive for benign process (RB), descriptive-neoplastic (RDN), descriptive-benign (RDB), indeterminate for neoplasm or benign process (RI), and purely descriptive (RD). Neoplasm, for the purposes of this study, included all primary and metastatic malignancies and lymphoma, as well as mucinous cystic neoplasms and endocrine tumors of the pancreas.

Results: 317 FNA (311 patients) from the kidney (26), liver (144), and pancreas (147) were identified that met our study criteria. The most common radiographic diagnosis was RDN (35.3%) followed by RN (30.6%), RD (10.7%), RDB (9.1%), RI (8.5%), and RB (5.7%). The overall positive predictive value (PPV) for a RN and RDN were 89.1% and 85.2%, respectively. The negative predictive values (NPV) for RB and RDB were 94.4% and 93.3%, respectively. CN diagnoses were 61.2% and CB diagnoses were 33.9% of the total FNA with a PPV of 100% and a NPV of 93.1%, respectively. Sampling error was the only observed false negative cytologic diagnosis (8 cases). CA (3.4%), CSN (0.6%), and CD (0.9%) were a minority of cytologic diagnoses.

Conclusions: Our study indicates that a radiographic diagnosis of an intra-abdominal mass or lesion continues to be descriptive more often then being called definitively benign or malignant. FNA continues to have a higher PPV over a radiographic diagnosis alone, however, the NPV for cytology and radiology appear to be identical in our series.

341 An Analysis of 961 Pericardial Fluid Samples Obtained over an 18-Year Period.

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Background: Malignant pericardial effusion is a relatively uncommon, but ominous complication of advanced malignancy usually seen in severely ill patients with multiple visceral metastases. The overall survival of patients with malignant pericardial effusion is primarily influenced by the extent and histopathology of their underlying primary malignancy.

Design: A computerized search of our LIS was performed for the period beginning July 1992 through June 2010 and all pericardial fluid cytology reports were reviewed. All correlating surgical pathology and relevant clinical information was extracted from a review of the electronic medical records of all of the malignant cases. The spectrum of different primary malignancies and prognostic significance of the malignant pericardial effusions was determined when adequate information was available.

Results: During this 18-year period, there were a total of 961 pericardial fluid samples accounting for 4% of all of the effusion cytology cases. Of the 961 cases, 828 were reported as benign (86%) and 133 (14%) were malignant. There were a total of 121 metastatic carcinomas (Table 1), 2 mesotheliomas, 9 hematologic malignancies and 1 germ cell tumor. The primary site for 80 metastatic malignancies (Table 2) included lung, breast, ovary, thymus, kidney, esophagus and pancreas. For the remaining 41 cases (34%), the primary site could not be established. The nine hematologic malignancies included 7 lymphomas and 2 myelomas. Almost all patients with follow-up information were treated with pericardial drainage or window, without chemotherapy instillation into the pericardium.

Table 1.

Types of metastatic carcinoma	Adeno-carcinoma	Non-small cell carcinoma	Poorly differentiated carcinoma	Squamous cell carcinoma	Small cell carcinoma	Thymic tumors
Number of cases	74	23	12	7	3	2
Percentage of cases	61%	19%	10%	6%	3%	2%

Table 2.

Primary sites	Lung	Breast	Ovary	Unknown primary	Others (kidney, thymus, pancreas, esophagus)
Number of cases	41	28	5	41	6
Percentage of cases	34%	23%	4%	34%	5%

Conclusions: Malignant pericardial effusions account for only a minority (14%) of all the pericardial effusions examined at our institution. Metastatic carcinomas account for more than 90 percent of these malignancies with lung and breast being the most common primary sites. However, for a significant percentage of patients with metastatic carcinoma (34%), the primary site of the metastatic carcinoma was unknown. While most patients did poorly, a few patients, surprisingly, survived many years following their diagnosis of a malignant pericardial effusion.

342 Cytologic Features and Diagnostic Yield of 330 Pancreatic Duct Aspirates.

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Background: Pancreatic duct aspiration cytology is routinely used as part of the evaluation of patients presenting with recurrent pancreatitis and is useful in the detection of pancreatic tumors involving the pancreatic ducts such as intraductal papillary mucinous neoplasm (IPMN).

Design: The pathology reports and clinical follow-up data for all available pancreatic duct aspirate cases for a 9½-year period were reviewed. The original cytologic diagnoses were grouped into the following diagnostic categories: unsatisfactory, negative for malignancy, extracellular mucin (without atypical cells), atypical cells suspicious for IPMN, IPMN, and adenocarcinoma. The presence of IPMN, pancreatic intraepithelial neoplasia, or adenocarcinoma was considered positive follow-up.

Results: There were 330 pancreatic duct aspirations. The mean patient age was 62.5 years (range: 25 to 87), 122 patients were men (37%), and 208 were women (63%). Specimen processing techniques included centrifugation and preparation of direct smears (231 cases), cytospin slides (2), Hettich preparations (18), ThinPrep processing (41), SurePath processing (37), and a combination of methods (1). Overall, 22 (7%) were considered unsatisfactory, 226 (68%) were negative, 25 (8%) contained extracellular mucin only, 29 (9%) were suspicious for IPMN, 10 (3%) were considered diagnostic of IPMN, 9 (3%) were atypical, and 9 (3%) were adenocarcinoma. Extracellular mucin only was reported for 17 direct smear (7%), 2 ThinPrep (5%), 3 SurePath (8%), and 3 Hettich (17%) cases. An abnormal diagnosis (either extracellular mucin, suspicious for IPMN, IPMN, atypical, or adenocarcinoma) was reported for 64 direct smear (28%), 6 ThinPrep (15%), 7 SurePath (19%), and 5 Hettich (28%) cases. Ninety cases (27%) had histologic follow-up: 73 were positive for neoplasm (81%) and 17 were negative (19%). There was 1 false positive (direct smear suspicious for IPMN, histologically only chronic pancreatitis). All 14 cases reported as extracellular mucin only had a neoplasm. If abundant thick extracellular mucin was also considered abnormal, the sensitivity was 58% for direct smears (33/57), 43% for ThinPrep (3/7), and 57% for SurePath (4/7).

Conclusions: An abnormal pancreatic duct aspirate cytology result is highly specific for neoplasia. The sensitivity of an abnormal cytologic diagnosis was 37%, but if abundant extracellular mucin was also considered an abnormal result the sensitivity improved to 56%.

343 Atypical Glandular Cells from Endometrial Carcinomas: Can Tumor Associated Inflammation Help?

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Background: Endometrial cancers are the most common gynecologic cancers in the United States. Often a diagnosis of AGC (atypical glandular cells) on PAP cytology proceeds biopsy confirmation of endometrial adenocarcinoma. Previous reports have noted the association of neutrophils with endometrial carcinoma on cytologic or histologic examination. However, a number of benign conditions also may show numerous neutrophils, such as endometrial hyperplasia, polyps, acute endometritis, and metaplasias. Characterization of tumor associated inflammation on Pap smears has not previously been studied in detail.

Design: 30 Pap smears, with a diagnosis of AGC and biopsy confirmed endometrial adenocarcinoma (EAC) on histology, were selected for examination. All adenocarcinomas were endometrioid type. For comparison, negative control cases consisted of Pap smears with a diagnosis of AGC and that had a concurrent, representative, negative biopsy. Each Pap smear slide was carefully examined by 2 boarded cytopathologists in order to quantitate and characterize the full spectrum of associated inflammation including: neutrophils, lymphocytes, eosinophils, histiocytes as well as the presence of blood/fibrin. Location of cells as intracellular or extracellular was noted. Cells counts were based on 10 HPF at 60x magnification.

Results: Cases of AGC with biopsy proven EAC, showed glandular clusters with multiple (3+), engulfed neutrophils in 22 (76%) of cases; whereas, cases of AGC with representative, negative (NEG) biopsies showed multiple (3+), engulfed neutrophils in no (0%) of cases. Presence of rare (1-3) engulfed neutrophils was similar for both, being present in 14% of AGC/EAC cases and 12% of AGC/NEG cases. The presence of blood/fibrin and other types of inflammatory cells on Pap showed no significant correlation with type of lesion on histology.

Conclusions: Atypical glandular clusters with multiple (3+) intracellular, engulfed neutrophils had the strongest correlation with biopsy proven endometrioid adenocarcinoma. This may prove to be an additional aid in determining when to favor neoplasia on Pap smears with atypical glandular cells.

344 Quantity Versus Quality: Predicting Malignancy in Salivary Gland Neoplasms.

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Background: Interpretation of salivary gland neoplasms has limitations because of overlapping morphologic features of benign and malignant lesions and a paucity of helpful ancillary tests. This study was conducted to determine if identifying specific morphologic features could improve diagnosis.

Design: Retrospective review was conducted of all salivary gland FNA from 01/01/05 to 06/01/09. Cases with at least one feature were flagged as "high risk" (HRFNA) regardless of diagnosis. Features were: increased cellularity, basaloid morphology, papillary fragments/morphology, irregular nuclear membranes, increased N/C ratio, mucin, prominent nucleoli, hyperchromasia, and coarse chromatin. Cases with myxoid stroma were excluded in the HRFNA evaluation.

Results: Out of 417 cases, 140 met criteria for HRFNA. For all cases (AC), 60% of false positives were due to over interpretation of a paucicellular specimen, and 64% of false negatives were from non-diagnostic or poorly sampled lesions. Characteristics for AC and HRFNA are in table 2. 60% of HRFNA with benign outcome had 1 "high risk" feature. 78.8% of cases with benign follow up (FU) had <4 high risk features. However, 46% of the cases with malignant FU had<4 features as well.

Table 1.Diagnostic characteristics

	ALL CASES	HRFNA
SENS	81.2%	97.6%
SPEC	89.3%	66.7%
PPV	96%	95.3%
NPV	60%	80%
CONCORDANCE	79.6%	90.6%

PPV=positive predictive value;NPV=negative predictive value;sens=sensitivity; spec=specificity

Table 2.Cases with high risk features and risk of malignancy

Feature (N)	Malignant on SP	Benign on SP
Coarse chromatin (11)	11(100%)	0(0%)
Pleomorphism(45)	41(91%)	4(9%)
Hyperchromasia (11)	10(90%)	1(10%)
Irregular nuclear membranes (43)	37 (86%)	6(14%)
High N/C (29)	24(83%)	5(17%)
Prominent nucleoli (56)	46(82%)	10(18%)
Cellularity (62)	43 (69%)	19(31%)
Mucin(8)	5(62.5%)	3(37.5%)
Basaloid morphology	3(25%)	9(75%)
Papillary morphology/groups (6)	(0)0%	6(100%)

SP=surgical follow up

Conclusions: 40% of HRFNA with malignant FU were diagnosed as a benign, low grade tumor, or atypical on FNA. Apparent improved sens and concordance in the HRFNA group was due to lower non-diagnostics or poorly sampled cases. Cellularity standards for interpretation may improve the diagnosis of salivary gland tumors. Cells with basaloid morphology were not at increased risk of malignancy in the absence of other malignant features. Number of features may not be as important as the specific feature and quality of the sampling.

345 Atypical Renal Tubular Cell Clusters in Voided Urine of Renal Transplant Patients Predict Graft Dysfunction: A Clinicopathologic Study.

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Background: We recently reported clusters of atypical cells in voided urine cytology in a significant minority of renal transplant patients (Arch Pathol Lab Med. 2010;134:1362). The three-dimensional, cohesive clusters, which occur only in transplant patients, are composed of cells with a high N-C ratio; round, hyperchromatic, eccentrically placed nucleus; prominent central nucleolus; and granular cytoplasm. The clusters could potentially be confused with urothelial, renal, or prostatic carcinoma. The cells show RCC+, CK 7+, p63- immunophenotype, consistent with renal tubular origin. The findings are transient, being absent in later urine cytology specimens from the same patient, but the clinical significance has not been examined.

Design: The voided urine cytology specimens for 100 renal transplant patients excluding polyoma virus infection were reviewed in our previous study. Clinical data was reviewed for these patients over a period of two years. Specifically, biopsy-proven acute graft rejection and episodes of significantly increased creatinine were noted.

Results: Of the 18 patients who exhibited atypical cell clusters; clinical follow-up was available in 17. Two (12%) patients had a concurrent biopsy showing acute rejection; 8 (47%) others developed biopsy-proven acute rejection in a mean of 3.8 months and maximum of 6 months, and another 5 (29%) developed other evidence of graft dysfunction (15 (88%) in total). Of the 82 patients without clusters, eighteen were lost for follow-up leaving 64 for evaluation; 2 developed biopsy-proven acute rejection and 2 developed other evidence of graft dysfunction (4 (6%) total). The presence of atypical renal tubular clusters had a sensitivity of 83% and specificity of 90% in predicting biopsy-proven acute cellular rejection, with a positive predictive value of 59% and negative predictive value of 97%. For evidence of graft dysfunction more broadly, the sensitivity was 79%, specificity 97%, PPV 88%, and NPV 94%.

Conclusions: A majority of renal transplant patients with atypical renal tubular cell clusters in voided urine developed acute rejection, and nearly all showed evidence of graft dysfunction in the months following identification of the abnormal cytology; findings which occurred very rarely in transplant patients without renal cell clusters. While further study is necessary to confirm these results, these findings preceded subsequent graft dysfunction and are likely associated with graft injury such as rejection.

346 Non-Hodgkin Lymphoma Diagnosis by Concurrent Fine-Needle Aspiration and Flow Cytometry: 123 Cases with Histologic Follow-Up.

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Background: Non-Hodgkin Lymphoma (NHL) diagnosis has traditionally been based on histopathologic analysis. With the increasing emphasis on non-invasive procedures in medicine, fine-needle aspiration (FNA) has become a common method of tissue acquisition. Despite studies suggesting that NHL can be accurately diagnosed using FNA in conjunction with flow cytometry (FC), its use is not completely accepted by either the hematopathology or hematology/oncology communities. Prior studies have

suffered from limited size, scope, or lack of histologic correlation. The purpose of this study was to assess the use of FNA with FC alone for NHL diagnosis using a large number of cases, all with histologic correlation.
Design: 123 FNA biopsies from 118 patients were analyzed. Each FNA had histologic follow-up and a diagnosis of NHL on the FNA and/or histology. Each FNA was assessed on-site for adequacy and a portion triaged for 6-color FC analysis for lymphoma using standard methods. Diagnosis of the FNA was rendered by a cytopathologist who had knowledge of the FC results. FC and histologic diagnoses were rendered by a separate group of pathologists. Accuracy of the FNA diagnosis was established by comparison to the histologic diagnosis and correlated with the body part biopsied, imaging method, and lymphoma classification.
Results: Of the 123 FNA biopsies, 88 (71%) matched the histologic diagnosis. Image-guided biopsies were more accurate (74%) than those performed on palpable lesions (57%). Imaging method had no effect on accuracy, but on-site adequacy assessment did correlate with accuracy (74% accuracy in adequate biopsies verses 64% in inadequate biopsies). Of the common FNA sites, bone and retroperitoneum had the highest accuracy (83% and 76%, respectively), while abdomen and neck were lower (68% and 55%, respectively). Of the common lymphoma classifications, plasma cell neoplasms and follicular lymphoma had the highest accuracy (83% and 77%, respectively); and B cell lymphoproliferative disorder, NOS, and diffuse large B cell lymphoma had the lowest (70% and 57%, respectively). Diagnosis of recurrence verses primary NHL had no effect on accuracy. Timing of the FNA diagnosis in relationship to the histology diagnosis had no effect on accuracy.
Conclusions: FNA with concurrent FC is an accurate method for NHL diagnosis. Image-guidance and on-site adequacy assessment improves accuracy.

347 The Utility of SOX-2 as a Marker of Squamous Differentiation in Fine Needle Aspiration Biopsy Material of the Lung.

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Background: Lung cancer is responsible for the most cancer related deaths worldwide. Introduction of new chemotherapeutic agents that are more effective in specific histologic subtypes of non small cell carcinomas has made distinguishing squamous from non squamous histologies in small biopsy specimens more important than ever in guiding clinical decision making. SOX2 is an embryonic transcription factor shown to be preferentially expressed in squamous cell carcinomas of the lung. We investigated the utility of SOX2 expression in distinguishing lung squamous cell carcinomas (SCC) from lung adenocarcinomas (ADC) in fine needle aspiration (FNA) cell blocks.
Design: Immunohistochemistry (IHC) was performed on sections from formalin fixed, paraffin embedded FNA cell blocks of 38 lung ADCs and 24 lung SCCs. SOX2 staining was evaluated for nuclear expression and graded from a scale of 1+ to 3+ and percentage positive cells. Tumors were graded as positive for SOX2 when greater than 10% of the cells showed nuclear staining. Embryonal carcinoma of the testis was used as a positive control.
Results: SOX2 IHC showed strong and diffuse nuclear expression in 67% (16/24) SCCs. The staining intensity for all SCC ranged from 2+ to 3+ with the majority showing 3+ staining. In contrast, a smaller fraction (24%, 9/38) of ADCs stained for SOX2, often with a weaker (1-2+) intensity and less number of tumor cells staining. The sensitivity of SOX2 for SCCs was 67% with a specificity of 76%, and a positive predictive value of 64%.
Conclusions: SOX2 expression is a specific marker for lung SCCs, especially when there is strong and diffuse staining. IHC for SOX2 is a valuable adjunctive marker in the diagnosis of SCCs. Due to its relatively high sensitivity and specificity, inclusion of SOX2 expression into the immunohistochemical panel of stains to distinguish lung SCC from lung ADC should be considered.

348 Comparison of Fine Needle Aspiration and Needle Core Biopsies in the Diagnosis of T-Cell Lymphomas.

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Background: T-cell lymphomas (TL) may be difficult to diagnose and subclassify on small biopsies. TL diagnosed by Fine Needle Aspiration (FNA) were reviewed, along with concurrently obtained lymph node core biopsies, to determine if FNA is a good diagnostic modality for diagnosing TL.
Design: Ninety-eight cases of TL, diagnosed at MD Anderson Cancer Center, were reviewed for a five year period, from 2005 to 2010. A subset of 23 cases were diagnosed by FNA and concurrent core biopsy, in conjunction with immunophenotyping by flow cytometry (FCM) and slide based immunohistochemistry (IHC). FCM results from FNA were incorporated into both core and FNA reports. The FNA results and core biopsy diagnoses for the 23 cases of TL were categorized by subtype, based on the WHO classification. The diagnosis by the two techniques was compared.
Results: All 23 concurrent cases (FNA and biopsy) showed a malignant T-cell lesion, however only 12 cases had concordant diagnoses and subclassification. These cases included 5 cases of Peripheral T-cell lymphoma-NOS (PTCL), 2 cases of PTCL-Lennert's variant, 2 cases of Anaplastic large cell lymphoma (ALCL) and the 3 cases of Mycosis fungoides (MF).
There were a total of 11 discordant cases. The 3 cases of CD 30 positive transformed PTCL were not identified by FNA but the diagnoses were rendered on the concordant lymph node core biopsy. The diagnosis of Angioimmunoblastic T-cell lymphoma (AILD) (5 cases) was also made by histologic analysis of the lymph node cores. Two cases of MF in transformation and the Extranodal NK T-Lymphoma were not able to be subclassified by FNA but were, subsequently, categorized on the concurrent lymph node core biopsy.
Conclusions: FNA is a good diagnostic tool, when compared to concurrent lymph node core biopsy, for PTCL, PTCL- Lennert's variant, ALCL and MF since there was complete concordance among the FNA diagnosis and lymph node core biopsy results. FNA aspiration failed to identify the cases of MF in transformation, CD 30 positive transformed PTCL and Extranodal NK T-Lymphoma. The diagnosis of AILD is also better rendered on the lymph node core biopsy, as opposed to FNA alone, due to the importance of the architectural features in this category of T-cell lymphoma. Use of immunophenotyping and morphology by FNA can result in a diagnosis of TL, however core biopsy was required for a more specific subtype.

349 Increased Epithelial Cell Abnormalities in Lymphocytic Cervicitis: A Five-Year County Hospital Experience.

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Background: In cervical Pap smears, lymphocytic cervicitis (LC) has a predominant finding of a polymorphic lymphoid population with tingible-body macrophages. LC has been shown to be associated with Chlamydia infection as well as atrophy. To date, no studies have been done to evaluate for associations with epithelial cell abnormalities (ECA) (i.e., dysplasia), or HPV or other infection.
Design: Records from a county hospital from 2005-2009 were searched for Pap smears with a diagnosis of LC. Pap smear reports were analyzed for additional findings such as ECA, atrophy, organisms, and hormonal status (premenopausal (PrM) vs. postmenopausal (PoM). Medical records were searched for concurrent HPV, GC, and Chlamydia testing. Results were analyzed by the study group as a whole, as well as by hormonal status group. Pap smear ECA, HPV, and Chlamydia rates for a female control population were evaluated as a whole, as well as by groups subdivided by age (<50 yrs, >50 yrs) as an estimate of hormonal status.
Results: Of the 143,158 Pap smears from 2005-2009, there were 283 (0.20%) cases of LC with 67 (23.7%) cases in PrM and 216 (76.3%) in PoM. An ECA was also present in 16.96% of cases, with a PrM ECA rate of 28.36% and a PoM ECA rate of 13.43%. The majority of ECAs were ASCUS (83.33%). The remaining ECAs included ASC-H, AGC, and LSIL, with LSIL more common in PreM than PoM. Of the 67 concurrent HPV tests performed, 11.94% were positive (PrM, 23.53%; PoM, 8.00%).
Of the 235 (83.04%) LC cases without an ECA, there were additional findings in 61 (16.0%). Of the 61 cases, 47 (77.1%) were atrophy and 14 (22.9%) were organisms. All cases of atrophy were in PoM. The 14 cases with organisms included Candida (71.4%), Trichomonas (21.3%), and Actinomyces (7.2%) species. Of the 101 concurrent GC/Chlamydia tests performed, 12 (11.88%) were positive for Chlamydia (PrM, 25.58%; PoM, 1.72%). All GC tests were negative.
The control group had an ECA rate of 19.13% (PrM, 21.22%; PoM, 10.39%). The control group HPV rate was 22.16% (PrM, 24.87%; PoM, 9.57%) and the Chlamydia rate was 5.51% (PrM, 5.78%; PoM, 0.44%).
Conclusions: Lymphocytic cervicitis is an uncommon finding in cervical Pap smears, more prevalent in postmenopausal women than premenopausal. Our study shows an increased incidence of concurrent epithelial cell abnormalities in Pap smears with LC, especially in premenopausal women (28.36%) when compared to control group premenopausal women (21.22%). Our findings also confirm the previously reported associations of LC with Chlamydia infection and atrophy.

350 Fine Needle Aspiration of Pancreatic Endocrine Neoplasm (PEN): An 18-Year Retrospective Study of 134 Cases.

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Background: PENs are relatively uncommon, accounting for 2-4% of all clinically detected pancreatic neoplasms and the long-term survival for these patients is much better than for patients with carcinomas of the exocrine pancreas. The preoperative diagnosis of PEN by FNA assists the surgeon in therapeutic decision-making.
Design: A search of our laboratory information system was performed from July 1992 through June 2010 to identify all cytology and surgical pathology cases. Patients with multiple endocrine neoplasia (MEN I) were excluded. Slides from cases with discrepancies between the cytologic and histologic diagnoses were re-examined.
Results: A total of 134 PEN cases diagnosed by FNA were collected over an 18-year period. The age of the patients ranged from 21 to 95 years with a mean age of 59 years. The male to female ratio was 1.3:1. The size of PEN ranged from 0.5 to 11.2 cm with a mean size of 3.2 cm. Fifteen cases had liver metastasis, 9 had lymph node metastasis and 5 had both liver and lymph node metastasis. Fifteen PEN cases were cystic lesions (11%), while 119 cases were solid lesions (89%). The FNA diagnoses were classified as follows: PEN (103 cases, 77%), suggestive of PEN (16 cases, 12%), no atypical cells identified (4 cases, 3%), non-diagnostic (7 cases, 5%), and other diagnoses (4 cases, 3%). Histologic correlation was available for a total of 78 FNA cases (58%). Among 56 cases diagnosed as PEN by FNA, 54 cases (96%) were confirmed histologically; the remaining 2 cases showed poorly differentiated adenocarcinoma with focal neuroendocrine features in one and no tumor in the other. Follow-up of the 9 cases diagnosed as suggestive of PEN by FNA, included 3 cases of PEN, 2 cases of chronic pancreatitis, 2 cases of ductal adenocarcinoma, 1 case of PEN vs solid-pseudopapillary tumor (SPPT), and 1 case of SPPT. There were 4 cases diagnosed as 'no atypical cells' and the 5 nondiagnostic cases that were proven to be PEN histologically. Finally, 4 other cases classified by FNA as adenocarcinoma (2), suggestive of carcinoma (1), and SPPT (1), respectively were diagnosed as PEN by histology.
Conclusions: Overall, 54 of the 70 histologically confirmed PEN cases (76%) were diagnosed correctly by preoperative FNA. The false negative rate, attributable to specimen hypocellularity and sampling error, was within acceptable limits (7%). Diagnostic pitfalls included SPPT, chronic pancreatitis and ductal adenocarcinoma. Despite its limitations, FNA of PEN remains a clinically useful procedure.

351 The Cytomorphological Evaluation of Large Cell Neuroendocrine Carcinoma of the Lung in Aspiration and Exfoliative Cytology.

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Background: Large cell neuroendocrine carcinoma (LCNEC) of the lung is a pulmonary neuroendocrine tumor that is considered to be an aggressive non-small cell carcinoma, and within the spectrum of neuroendocrine tumors in the lung, which includes carcinoid, atypical carcinoid and small cell carcinoma. The correct diagnosis of LCNEC in cytologic specimens is important for prognosis and management. Although the histologic criteria for LCNEC of the lung are well established, studies on the cytomorphological features are limited.

Design: The surgical pathology files were searched for pulmonary LCNEC from January 2001 to September 2010. From a total of 54 cases histologically diagnosed as pure LCNEC, 27 patients had cytological evaluation before surgical excision and were included in this study. The cytologic specimens included 12 fine needle aspiration (FNA) biopsies and 15 bronchial brush (BB) or bronchial wash (BW) specimens. The available cytologic specimens were reviewed by two cytopathologists.

Results: 16 patients were men and 11 were women. The mean age was 62.2 years ranging from 44 to 86 years. Original cytologic diagnoses of all 27 patients were as following: 3 LCNEC, 3 non-small cell carcinomas (NSCLC), 4 small cell carcinomas, 5 poorly differentiated carcinomas, 5 atypias, and eight negatives. 10/12 (83%) FNA biopsies were reported as positive for malignancy. In contrast, only 4/15 (27%) BW/BB specimens were reported as positive for malignancy. 14 cytologic specimens diagnosed as positive were available for review. Variable cytologic features are listed in Table 1.

Table1 Cytomorphologic features of 14 positive cytologic specimens

Cytomorphological features	No. of Case	%
Necrotic background	9	64
Both clusters and single cells	14	100
Pleomorphism (moderate to marked)	14	100
Nuclear size (inter-large)	14	100
High N/C ratio	14	100
Crush artifact	8	57
Coarse chromatin	7	50
Nuclear molding	12	86
Nuclear Palisading	5	36
Rosette-like structure	9	64
Marked mitotic activity	2	14
Prominent nucleoli	3	21

Conclusions: Our results illustrate that LCNECs are uncommon NSCLCs that can be difficult to evaluate in cytological material, particularly in exfoliative or brushing cytology. However, in a tumor of non-small cell type without definitive squamous or glandular differentiation, the presence of intermediate to large nuclei, marked pleomorphism, high N/C ratio, and nuclear molding, one should consider incorporation of neuroendocrine markers in the immunopanel, which may enhance the ability to identify these cases. This is important given the poor prognosis of LCNEC carcinomas within NSCLCs, and the potential for tailoring the treatment for these patients.

352 Utility of Double Immunostaining for P16ink4a and MIB 1 (Ki-67) in Identification of HSIL Cells in Women with Preliminary ASCUS Pap Test Results.

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Background: ASCUS)/LSIL Triage Study have established adjunctive high risk HPV testing provides cost-effective basis for triage to colposcopy for women with ASCUS Paps who test HPV positive (+) versus return to routine screening for those who test HPV negative. HPV (+) ASCUS cases still have very limited positive predictive value for identifying women with underlying high grade CIN2/3. P16 is strongly over-expressed in nearly all high-grade pre-cancerous and malignant cervical lesions, detection of P16 over-expression has been proposed as a surrogate marker for the transforming activity of high-risk HPV. In order to assess the hypothesis that detection of individual cells simultaneously co-expressing P16 protein and proliferation marker Ki-67 can be used as an indicator for presence of CIN2/3, ina large prospective study on cervical cytology specimens, the aim of our study was to identify HSIL cells in women with preliminary ASCUS Pap test results.

Design: Residual fluid from liquid-based ThinPrep™ cytology specimens of women attending cervical cancer screening at a major tertiary hospital over a one year period were used for the analysis. Specimens with any abnormal Pap cytology result (ASC-US+) were included. For each case, an additional LBC slide was prepared and immuno-stained using a prototypic dual staining reagent kit (**CINtec®Cytology, Dual stain**) for the simultaneous detection of P16 and Ki-67 expression on the same slide. The presence of one or more individual cells co-expressing both P16 and Ki-67 were interpreted as "positive" test result. Follow-up biopsy and HPV results were obtained.

Results: A total of **1396** ASCUS Pap tests were assessed during the study, for which histopathologic follow-up has been available to date in **541** cases. Dual stain cells were identified in 15% of ASCUS Pap tests. Sensitivity of the Dual stain was 87.0% for CIN2+ and 95.7% for CIN3+, with specificity of 89.8% for non high-grade CIN. In ASC-US cases with positive HPV results, a positive Dual stain result identified all CIN3+ cases at high levels of specificity (up to 80%).

Conclusions: 1. Initial results from our cytology specimens subjected to simultaneous p16/Ki-67 dual staining and with biopsy follow-up indicate both high sensitivity and specificity for this novel screening approach to detection of histopathologic CIN2/3+. **2**. Results showing high specificity rates for the dual stain support this approach as a promising adjunctive immunocytochemical test for detection of underlying histopathologic CIN2/3+.

353 BRAF Analysis in Fine Needle Aspiration Biopsy (FNAB) of Papillary Thyroid Carcinoma (PTC).

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Background: BRAF mutation is the most common genetic alteration in papillary thyroid carcinoma (PTC) and has been associated with extrathyroidal extension, metastases and recurrence. It has been reported to be an independent prognostic factor that can be used to preoperatively identify PTC patients with aggressive disease. Thus, it may be useful in tailoring the initial surgical extent for these patients. Our objectives were to evaluate the BRAF status using archived cytology specimens from patients with histologically proven PTC and long term follow up and to correlate these results with the original cytology diagnosis, clinicopathological stage at surgery and long term outcome of these patients.

Design: Fine needle aspiration biopsy (FNAB) material from 24 cases of PTC with corresponding confirmatory thyroidectomy specimens and more than 10 years follow-up were used in this study. The slides were evaluated to confirm the presence of diagnostic cells and DNA was extracted from the previously-stained cytology slides. Standard PCR was performed for an amplicon in BRAF exon 15 that included the V600E mutation site. Locked nucleic acid (LNA) PCR was also performed to increase sensitivity. Cycle sequencing was performed using the BigDye Terminator kit and analysis was performed on an ABI automated sequencer (Applied Biosystems). The forward and reverse sequences were analyzed for point mutations.

Results: The frequency of BRAF mutation in PTC in our series was 38%. The correlation of the BRAF status with the cytology diagnosis, clinicopathological stage at surgery and recurrence are summarized in Table 1. Notably, only one patient died of disease. This case was BRAF(+), tall cell variant PTC, presented at high stage, developed recurrence and distant metastases. Eight of the nine (89%) BRAF(+) cases were of the tall cell and classical variants while 5 of the 15 (33%) BRAF(-) cases were of the follicular variant.

Table 1. BRAF status and correlation with original cytology diagnosis, clinicopathological stage at surgery and recurrence.

BRAF Status	PTC (N=24)	FNAB Diagnosis			Clinicopathologic Feature	
		Positive [N=11/24 (46%)]	Suspicious [N=6/24 (25%)]	Atypical [N=7/24 (29%)]	> stage 1 [N=6/24 (25%)]	Recurrence [N=4/24 (17%)]
BRAF (+)	9 (38%)	8 (73%)	1 (17%)	0	4 (67%)	2 (50%)
BRAF(-)	15 (62%)	3 (27%)	5 (83%)	7 (100%)	2 (33%)	2 (50%)

Conclusions: BRAF mutation seems to be associated with higher clinicopathological stage at surgery but it is not predictive of recurrence in the long term follow up in this series. BRAF mutation status analysis did not increase the accuracy of thyroid FNABs diagnosed as suspicious or atypical.

354 The Diagnostic Value of Cell Block from Liquid-Based Bronchial Washings Cytology Specimens in the Diagnosis and Subclassification of Pulmonary Neoplasms.

G Collins, S Zhang, J Thomas. Louisiana State University Health Sciences Center-Shreveport, Shreveport.

Background: Though cell block (CB) preparations of bronchial washings cytology specimens are routinely done in our institution, its diagnostic value has received little attention in the literature. The diagnostic value of routine CB as adjunct to liquid based (ThinPrep) bronchial washing cytology preparations (LBC) in the detection and subclassification of pulmonary neoplasms has not been evaluated. This study aims to evaluate the diagnostic utility of CB in this setting.

Design: This study included all 74 bronchial washings samples and CB diagnosed as malignant or suspicious/atypical from bronchoscopies done in our institution during 2009. 28 randomly selected negative cases were also reviewed. The LBC and CB preparations were reviewed independently. Deeper levels, ancillary stains, and PCR analysis were done on CB when necessary for specific classification of tumors. LBC and CB diagnoses were compared and correlated with final histology/bronchial brushing diagnoses.

Results: LBC and CB yielded adequate material for diagnosis in all cases. Table 1 shows the correlation of CB and LBC diagnoses.

Table 1: Comparison of LBC and Cell Block Diagnoses

LBC diagnosis (# of cases)	Cell Block diagnosis (# of cases)			
	Positive (29)	Suspicious (9)	Atypical(26)	Negative (38)
Positive (18)	11	2	5	0
Suspicious(13)	7	4	1	1
Atypical (41)	10	2	20	9
Negative (30)	1	1	0	28

The use of cell blocks increased the number of positive malignant diagnoses from 18 (with LBC diagnosis only) to 36, with an increase in diagnostic yield of 100 %. CB detected more malignant neoplasms (29) than did LBC (18). Specific tumor diagnosis was possible in 22/29 (76%) malignancies detected by CB compared with

12/18 (67%) detected by LBC. Bronchial brushings done in 35/36 (97%) malignant cases confirmed malignancy in 32 cases. Similarly, histology confirmed malignancy in 33/36 (92%) cases.

Conclusions: The use of CB preparation significantly improved diagnostic yield in this study. In addition, specific diagnosis of tumor type was often possible, and material was available for immunohistochemical and molecular studies when necessary. In summary, the use of CB on bronchial washings as an adjunct to LBC is useful in the diagnosis of pulmonary neoplasms especially in LBC suspicious and atypical diagnostic categories.

355 Significant Cervical Squamous Lesions (CIN2/3) in the Follow-Up of Women with Liquid-Based (Surepath) Pap Tests Interpreted as Atypical Cells of Undetermined Significance: Impact of Changes in Diagnostic Criteria and HPV Vaccination.

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Background: The main purpose of identifying atypical squamous cells of undetermined significance (ASCUS) in Pap tests is to identify and treat potential underlying significant cervical carcinoma precursor lesions (CIN2/3).

The aim of this study was to determine the impact of the Bethesda 2001 redefinition of ASCUS and the advent of HPV vaccination on the HPV genotypes and follow-up biopsy diagnoses of CIN2/3 of women diagnosed as ASCUS in our low-risk, relatively older, predominantly suburban screening population.

Design: Cases diagnosed as ASCUS that had a reflex HPV DNA test performed by PCR from 01/01/2001 to 6/30/2001 were identified and histologic diagnoses on biopsies performed within 6 months of the Pap diagnosis were collected. The study period was divided into 3 periods, P1=1/1/2001-12/03/2002, the date of the introduction of B2001 in our institution, P2=12/04/2003-6/30/2006, the date of FDA approval of the HPV vaccine Gardasil(r) and P3=07/01/2006-6/30/2009.

Results: There were a total of 18523 patients identified with an ASC-US diagnosis during the study period. The mean age for the 3 groups was 36.51±12.91, 35.14 1±12.89 and 35.52 ±13.76.

	ASC-US	any HPV type (% of all ASC-US)	hr-HPV+ (% of all ASC-US)	HPV16/18 (% of hr-HPV)	Biopsy Rate (% of all ASC-US)	CIN1 (% of all Bx)	CIN2/3+ (% of all ASC-US)
P1	2028	552 (27.2%)	277 (13.6%)	207 (74.7%)	529 (26.0%)	132 (24.9%)	68 (3.4%)
P2	8636	3215 (37.2%)	1496 (17.3%)	789 (52.7%)	2083 (24.1%)	628 (30.1%)	248 (2.9%)
P3	7859	3284 (41.7%)	1666 (21.2%)	721 (.43.2%)	1235 (15.7%)	359 (29.0%)	208 (2.6%)
p value*		1,2,3 = <.001	1,2,3 = <.001	1,2,3 = <.001	1=.04, 2,3=<.001	1=.018, 2=.2, 3=.5	1=.2, 2=.09, 3=.4

* 1 =P1 vs P2; 2 = P1 vs. P3; 3 = P2 vs. P3

During the study period there was a drop of the overall ASC-US rate and ASC/SIL ratio due to our laboratory's efforts to reduce these rates. While the overall HPV16/18 rates changed little from period to period, the %HPV16/18 of all hr-HPV types declined significantly.

Conclusions: Our results show that despite a significant increase in overall and hr-HPV rates observed between the periods, the follow-up CIN2/3 rates showed a modest decline instead of the expected increase. This was most likely due to the decrease of HPV16/18 fraction of hr-HPV brought about by the exclusion of ASC-H from ASCUS from P1 to P2 and the effect of vaccination from P2 to P3.

356 Comparison of Specimen Processing Methods for Biliary/Hepatic Duct Brush Specimen FISH Interpretation.

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Background: FISH (fluorescence in situ hybridization) is increasingly being used in conjunction with routine cytology on biliary/hepatic duct brushing specimens for increased sensitivity in the diagnosis of cholangiocarcinoma and ductal dysplasia. For FISH specimens to be adequately analyzed a preparation must be made that presents the cellular component without overlap of cell groups. The aim of this study was to establish a standardized process for FISH preparations of consistent, diagnostic quality.

Design: Various processing methods were utilized to process biliary duct brushing and hepatic duct brushing specimens (n=45) in order evaluate signal interpretability in FISH. FISH assay evaluated abnormalities on chromosomes 3, 7, 17 and 9p21 (UroVysion, ™ Abbott Laboratories). Biliary duct/hepatic duct brushings collected endoscopically were submitted in CytoLyt ® for routine cytology as well as FISH. Different processing techniques included: Shandon Cytospin® (Thermo Fisher Scientific) preparations (n=6); air-dried smears (n=4); cell block specimens (n=4); ThinPrep® (Hologic™) specimens (n=5) and ThinPrep® specimens (n=26) preceded by a blending step (Waring Blender™). The overall success and failure of the various processing modalities was examined.

Results: Cytospin® preparations were diagnostic in three cases (50.0%). Overlapping of cells made the signal evaluation problematic. Four specimens (100%) processed as air-dried direct smears failed due to lack of adequate cellularity. One ThinPrep® specimen (17.0%) failed due to cellular overlap. Four specimens from formalin-fixed cell block material (100%) failed for insufficient material. Twenty-six specimens blended with a Waring Blender® for 20 seconds prior to ThinPrep® processing (100%) were successfully processed for FISH.

Conclusions: The characteristic properties of biliary /hepatic duct brushing specimens are problematic in creating a mono-layer preparation for FISH preparations.Teasing the cellular material from the endoscopic brush results in flecks or strings of tissue that when processed using the ThinPrep® processor often creates cellular overlap. To reduce this clumping of cellular material we have found that a brief (20 second) blending of the specimen in a laboratory issue Waring Blender® breaks up the cellular clumps without distorting the cellular morphology or affecting the results of FISH.

357 How Much Is Enough? Adequacy in Pap Tests after Hysterectomy and Radiation.

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Background: We identified a number of patients with unsatisfactory Papanicolaou (Pap) tests (UPTs) despite multiple aggressive scapula scrapings. These patients tended to be older and post hysterectomy and radiation/chemotherapy. The 2001 Bethesda System minimal criteria for a satisfactory Pap from liquid-based preparation is 5000 well-visualized/well-preserved squamous cells. In cases of post hysterectomy, however, Bethesda states,"laboratories should exercise judgment in reporting cellularity based on the clinical and screening history," and "lower cellularity may be acceptable under these circumstances." No minimum cellularity has been proposed by Bethesda or in the literature, and we propose a number to standardize adequacy reporting in these patients.

Design: We searched our data for patients >40 years old with ThinPrep UPTs from January 2007 to January 2009 and recorded demographics, clinical diagnoses, and history of hysterectomy and radiation/chemotherapy. Vaginal samples were collected using a cytobrush/spatula, rinsed in PreservCyt transport medium, and processed by ThinPrep T2000 processor (Cytyc Corporation, Marlborough, MA). UPTs with subsequent negative for intraepithelial lesion (NIL) on follow-up were studied to determine cellularity.

Results: 4019 Paps were performed on women >40 years of age over 2 years, 98 (2.4%) of which were unsatisfactory. 27/98 (28%) patients had previous hysterectomy and radiation/chemotherapy for gynecological malignancy. 23/27 (85%) had insufficient cellularity and 4/27 (15%) were obscured by blood or inflammation. 24 (89%) patients had follow-up Paps. 20 (83%) were diagnosed as NIL, 2 (8.7%) unsatisfactory, and 2 (8.7%) ASC-US. Cellularity varied considerably, ranging up to 3000 epithelial cells per slide.

Conclusions: Prior studies have shown that UPTs carry a higher risk of significant histologic abnormalities on follow-up than negative satisfactory Paps. However, these studies did not exclusively investigate our specific patient population, rather examined all Paps performed. Our study showed a low incidence of abnormal follow-up findings. Bethesda minimum criteria (5000 cells) for cervical cytology can be difficult to reach in instances of advanced age, total hysterectomy, and chemo or radiation treatment. Our results show that fewer epithelial cells can be considered adequate, and immediate repeat pap tests are unnecessary. A minimum cellularity should be set in order to reduce variability among evaluations of adequacy, and we propose 2000-3000 cells should be adequate in this population.

358 Evaluation of HPV Positivity Rate as a Quality Assurance Measure for a Cytopathology Lab.

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Background: With the advent of validated techniques for HPV testing, a new metric to monitor the accuracy of ASCUS(Atypical Squamous Cells of Undetermined Significance) diagnoses has been introduced. An application of this metric in our lab with a mixed demographic offers a new perspective to the existing studies. The CAP QProbes study of 68 institutions classifies HPV positivity rates for ASCUS cases by different demographic metrics including type of institution and patient age. The overall HPV positivity rate for their study, including teaching and non-teaching institutions, is 43.74% (SD of 17.77%).

Design: We compared the results of 814 reflex HR-HPV(High Risk HPV) tests over a 6 month period in 2010 for ASCUS diagnosis cases to the national data reported by the ALTS trial and the CAP QProbes study. The purpose of this comparison was to evaluate whether our mixed patient demographics contribute to the deviation in our HPV positivity rate.

Results: 1. Table below shows that the HPV positivity rates (**HPR**) of university clinics(UC) and suburban/peripheral clinics (PC) cases deviate from the ALTS and CAP QProbes study as well as from each other.

Positivity Rate by Institution

	UC	PC	WTD AVG UC/PC	ALTS Trial	CAP QProbes study
HPV positivity rate %	51.5	25.2	29	50.6	43.74
# of cases	127	687	814	3488	68 institutions

HPR in % calculated as: (# of HPV (+) cases / Total # of ASCUS cases) x100

2. Age distribution of the two populations is not uniform. 35% of the UC cases are within the 21-25 age group compared to only 15 % for the PC. The UC mean age is 32 (median of 28, SD of 11). The PC mean age is 38 (median of 37, SD of 13)

3. Comparison of **HPR** for 4-year categories of ages (<=20, 21-30, 30-40, 40-90) for UC, PC, the combined UC/PC entity, and CAP QProbes show that the **HPR** trends lower in an almost linear fashion up to age 40 and then stabilize or decrease slightly in most cases.

Conclusions: Our study evaluated a new parameter- impact of demographics on **HPR** which is in addition to the parameters reported in CAP QProbes. The results show that our institutions (UC and PC), in spite of the influence of demographics lie within 1 SD of the CAP QProbes study (acceptable range suggested by CAP QProbes is 2SD). Although this degree of variability is acceptable, we want to explore if **HPR** in individual hospital settings can be utilized to achieve a more precise QC and can serve as a tool to monitor individual pathologist performance.

359 Is High Grade Cervical Intraepithelial Neoplasia Ever Found on Follow Up of Equivocal and Low Positive Hybrid Capture 2 Results in Women 50 Years and Older?

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Background: The Hybrid Capture 2 (HC2) test interpretation is currently the same for women of all ages despite the known age-dependent variation in prevalence of

high-risk human papillomavirus (HPV) infection. Manufacturer's guidelines define an equivocal result as a Relative Light Unit/ Cut Off (RLU/CO) value of 1-2.5. The high grade cervical intraepithelial neoplasia (CIN2+) risk of older women with specimens in this range, as well as those with a low positive result (RLU/CO ratio of 2.5-10), has not been determined in our population.
Design: We identified all specimens from women over 50 years old with an equivocal or low positive HPV test result at our institution during a 13 month period from June 2009 to July 2010. These index Pap smear reports and their follow up cytology and histology reports were retrieved. All cases with atypia or CIN of any grade in these follow up specimens were reviewed at the microscope by two pathologists with expertise in cervical pathology and cytopathology.
Results: 102 equivocal (46, 45%) or low positive (56, 55%) HC2 tests on women >50 were identified. Index Pap smear reports were found for 88 cases and consisted of 37 NILM, 43 ASCUS, 2 ASC-H and 6 LSIL. Follow up specimens were available for 49 cases (48%) including 30 Pap smears and 33 cervical biopsies or endocervical curettages. Follow up cytologic specimens were 11 NILM, 4 ASCUS and 1 LSIL. 13 biopsy/ curettage specimens were negative and 6 showed CIN1 or postmenopausal squamous atypia. 14 cases had both cytology and histology follow up; 5 NILM/Negative, 4 NILM/ CIN1, 3 ASCUS/Negative, 1 LSIL/VAIN1 and 1 case was reported as ASCUS/CIN2. This latter case was classified as CIN1 after review including additional p16 and Ki67 IHC, it was also negative for HPV by in situ hybridization. Therefore, none of the 33 cases with histological follow up and 49 cases with histological or cytology follow up showed CIN2+. Of 24 cases with ASCUS cytology which underwent colposcopy, none showed CIN2+.
Conclusions: The absence of CIN2+ in women >50 with equivocal or low positive HC2 results suggests that the ideal laboratory interpretation and ultimate reporting of this test could be age specific. Older women could be spared from unnecessary follow up colposcopies and biopsies while maintaining high test sensitivity. We recommend the HC2 RLU/CO threshold levels be raised for older women undergoing reflex HPV DNA testing after ASCUS cytology on Pap smear.

360 Relative Utilization of Interventional Radiology Guided Fine Needle Aspiration Biopsy and Core Needle Biopsy for the Investigation of Renal Masses.

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Background: Renal masses can be sampled either by fine needle aspiration (FNA) or by thin core needle biopsy (CNB). In a state of the art institution where interventional radiology and pathology support is available including immediate assessment, we undertook this study to evaluate the relative utilization and efficacy of both these techniques in the preoperative investigation of renal masses.
Design: We searched our pathology files from January 2009 to December 2009 for patients with renal masses who were subjected to FNA and CNB, only FNA or CNB. Pathological parameters reviewed: size of the lesion, diagnosis on FNA/CNB and any ancillary techniques performed on both the types of specimens. Findings were correlated with surgical resection or clinical follow up. The relative sensitivities of FNA and CNB were compared by McNemar's test.
Results: We studied 179 patients; 145 underwent FNA and CNB, 20 FNA and 14 CNB alone. In the former group, a definite diagnosis was rendered on FNA in116 (80 %) patients in comparison to 134 (92 %) on CNB. While FNA alone was diagnostic of malignancy in 6/145 (4%) patients, CNB alone was positive in 22/145 (!5%) of the patients. The results of FNA alone performed in 20 patients yielded concordant result in all patients (100%) (16 malignant and 4 benign cysts,); CNB alone performed in 14 patients yielded concordant results in 13/14 (93 %) patients. Ancillary immunohistochemical studies were performed predominantly on CNB specimens in comparison to FNA (50 % vs 10 %).
Overall definite diagnosis was rendered on 127/156(81%) patients by FNA and in 134/148 (91%) patients by CNB. The sensitivity of CNB was significantly better than FNA using surgical resection and/or clinical follow up as the gold standard, (p=0.0137),
Conclusions: 1.FNA was utilized more often than CNB for the initial investigation of renal masses 2. We found CNB to be significantly better than FNA for the investigation of renal masses. 3. Although both techniques can be utilized for rendering a definite diagnosis, it is prudent to procure tissue using both modalities if possible due to the chances of discordant results using either technique alone.4. While both CNB and FNA can yield definite results based on conventional morphological examination, CNB was preferred over FNA in cases requiring ancillary studies for rendering a definite diagnosis.

361 Relative Utilization of Interventional Radiology Guided Fine Needle Aspiration Biopsy and Core Needle Biopsy for the Investigation of Lung Lesions.

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Background: Initial investigation of lung lesions is generally performed using fluoroscopy or computed tomography guided fine needle aspiration biopsy (FNA) or core needle biopsy (CNB). In a state of the art institution where interventional radiology and pathology support including immediate assessment was available, we undertook this study to evaluate and compare the current utilization and efficacy of FNA and CNB for the investigation of lung lesions.
Design: We searched our pathology files from Jan 2009 to Dec 2009 for patients who underwent investigation of their lung lesion by FNA and CNB or by only FNA or CNB. Pathological parameters reviewed were : size of the lesion, diagnosis on FNA/ CNB and ancillary techniques including immunostaining (IHC) and molecular tests (MT) performed using both these techniques. Results were compared with surgical resection and/or clinical follow up and sensitivity of both techniques determined by McNemar's test.
Results: We studied 740 patients who underwent FNA and CNB, 153 FNA only and 84 CNB only. In the former group, using surgical resection and/or clinical follow up as the gold standard, a definite diagnosis was rendered in 651/740 (88%) (Benign-144, Neoplastic-507) by FNA in comparison to 695/740 (94%)(Benign-172, Neoplastic-523) by CNB. A total of 153 patients underwent FNA only which yielded definite results in 143 (93.5%) (Benign-40, Neoplastic-103). All the 84 pateints who had CNB only yielded definite result 100% (Benign-35, Neoplastic-49). Ancillary studies were performed more often using CNB than by FNA; IHC: 40% vs 11%; MT: 16% VS 4%.
Overall, a definite diagnosis was rendered by FNA in 794/893 patients vs 779/824 and their sensitivities, 89% vs 94.5% were not statistically significant. The non-diagnostic rate of FNA was however higher than CNB ;99/893 (11%) vs 45/824.(5.5%).
Conclusions: 1.FNA was utilized more often than CNB for the investigation of lung lesions.
2.The sensitivity of FNA was comparable to that of CNB (89% vs 94.5%).
3. Ancillary studies were performed more often using CNB in comparison to FNA.
4.Non-diagnostic result by FNA was more often encountered than by CNB in this study,
5.Although either technique can be used alone, because of the possibility of discordant results, performance of both techniques if possible can ensure a definite result.

362 Interobserver Agreement Using the New Bethesda System for Reporting Thyroid Cytopathology.

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Background: The recently proposed Bethesda System for Reporting Thyroid Cytopathology provides a uniform system for reporting the results of thyroid fine needle aspiration (FNA). Six diagnostic categories correspond to increasing risk of malignancy. The first three categories (non-diagnostic or unstatisfactory, benign, and atypia of undetermined significance or follicular lesion of undetermined significance) have lower risks of malignancy and are managed by clinical follow up or repeat FNA. The latter three categories (follicular neoplasm or suspicious for a follicular neoplasm, suspicious for malignancy, and malignant) have higher risks of malignancy and are clinically managed by surgical lobectomy or near-total thyroidectomy. The objective of this study was to report interobserver diagnostic variability. The precise agreement was evaluated as was practical agreement based on segregation of categories in two groups based on clinical management, i.e. clinical follow up-repeat FNA vs. surgical excision.
Design: A computer search of all thyroid lesions diagnosed by FNA from Jan 2008 to Dec 2009 was performed. These cases were reviewed independently by three pathologists and classified using the Bethesda System. Each category was given a gradient from 1-6 beginning with 1: Non-diagnostic, 2: Benign, 3: Atypia of undetermined significance, 4: Follicular Neoplasm, 5: Suspicious for Malignancy, and 6: Malignant. The pathologists were blinded to the clinical data, prior cytology diagnosis and subsequent surgical pathology findings. Interobserver variability was studied with respect to an precise agreement among all three pathologists and practical agreement in categories 1-3 vs 4-6, which would result in different patient management.
Results: A total of 79 cases classified utilizing the Bethesda System showed the following results: only 39.2% (31/79) cases had perfect agreement, but 65.8%(52/79) had practical agreement. The majority of cases with perfect agreement 64.5% (20/31) were for the benign category. Categories with the least absolute agreement include atypia of undetermined significance, follicular neoplasm and suspicious for malignancy each with only one case of absolute agreement 3.2% (1/31). A mean maximum difference of raters over 79 cases is 1.06+/- SE 0.11($p < 0.01$).
Conclusions: Not surprisingly, there is poor agreement of specific diagnostic categories among pathologists. Practical agreement relating to patient management is significantly better. Diagnostic agreement is likely to improve with increasing experience in the use of the Bethesda System.

363 Impact of Eight-Color Flow Cytometry on the Diagnosis of Cerebrospinal Fluid (CSF) Involvement by Leukemia or Lymphoma.

JD Duven, CJ Holman, T Singleton, AM Cioc, DM McKeon, SE Pambuccian. University of Minnesota, Minneapolis.
Background: Cytologic examination (CYT) of the CSF has long been the standard diagnostic procedure for detection of leukemia and lymphoma (L&L) in the CSF. However, recent reports have shown an increase in the detection rate of CSF involvement by L&L ranging from 20% to 600% by the concomitant use of flow cytometry (FC). The aim of this study was to determine the impact of 8-color FC, a method ideally suited for hypocellular samples such as CSF, on the diagnosis of CSF involvement by L&L.
Design: All cases that had CYT and FC performed on samples obtained from the same lumbar puncture from 1/1/08 to 8/1/10 were identified. CYT and FC diagnoses were independent, the CYT diagnosis usually preceding the FC interpretation. 8-color FC was performed on a BD FACS Canto II flow cytometer. The typical antibody panel consisted of CD5, CD10, CD19, CD20, CD45, κ, λ and either CD14, CD38, CD56 or CD58 depending on the L&L suspected, with a secondary T-cell or myeloid panel performed as needed. Age and sex of the patient, L&L type, CYT and FC interpretations were entered into a spreadsheet for analysis.
Results: We identified a total of 282 cases from 143 patients. 78M/65F, aged 4-84 (mean 51) with diagnoses of acute lymphocytic leukemia (n=23), acute myelocytic leukemia (n=28), acute bilineage leukemia (n=1) chronic lymphocytic leukemia (n=4) chronic myelocytic leukemia (n=3) hairy cell leukemia (n=1), lymphoma (n=57), and

other diagnoses (n=26). Of the 19 CYT+ cases, 8 were lymphomas and 11 leukemias (AML, ALL, CLL, CML); the FC+ cases were 9 lymphomas and 23 leukemias (AML, ALL, CML).

Comparison of Flow Cytometry and Cytology Results

	FC Negative	FC Atypical	FC Suspicious	FC Positive
CYT Negative	214	2	9	13
CYT Atypical	13	1	4	1
CYT Suspicious	1	0	1	2
CYT Positive	1	0	2	16

84% of CYT+ cases were FC+; 50% of FC+ cases were also CYT+; The use of FC resulted in the diagnosis of 12 additional cases, corresponding to an increase in identification of CSF involvemnt by L&L of 168%. However, FC was negative, atypical, or suspicious in 3 of the CYT+ cases.

The sensitivity, specificity, PPV, and NPV for CYT+ as compared to FC+ were: 0.50 (0.38-0.58), 0.98 (0.96-0.99), 0.76 (0.58-0.89), and 0.94 (0.92-0.95), respectively.

Conclusions: FC aids in the identification of CSF involvement by L&L and should be used in conjunction with CYT whenever CSF involvement by L&L is suspected. Our data suggests that a management algorithm which would only perform FC when CYT findings are atypical or above would miss a significant number of cases with L&L involving the CSF.

364 Gynecologic Telecytology Using Automated Local Image Selection with Remote Interpretation: The Results of a Phase 2 Prospective Trial.

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Background: Internet-based telecytology would allow for remote interpretation of cervical cytology specimens and has the potential to facilitate regionalization. We have previously shown that remote automated field-of-view (FOV) image selection and transmission can allow for successful interpretation of cervical cytology cases (Am J Clin Pathol 2008;12:686). To test the feasibility of this method in a typical high risk patient population a prospective study was initiated.

Design: Human subjects approvals were obtained. Patients were consented and prospectively enrolled from 2 colposcopy clinics – one collecting Thin Prep cervical cytology specimens and the other SurePath (SP) samples. Slides were scanned by a customized FocalPoint device, the 30 highest risk FOVs were identified, and low resolution images were automatically captured and transmitted to a customized remote reading station. Images were interpreted by a single observer and categorized as normal or abnormal for triage. Interpretations in the test arm were compared to the original glass slide results made as part of each patient's routine care.

Results: 274 patients were enrolled (155 TP, 119 SP). The overall sensitivity of a positive case (ASC-US or greater (+)) triage was 85% with a specificity of 67%. The positive predictive value (PPV) was 44% and the negative predictive value (NPV) was 93%. For LSIL+ and HSIL+, the sensitivities were 79% and 100%, respectively. When examined by specimen type, SP performed better than did TP with sensivity/specificity for ASC-US + of SP being 92%/66% and for TP 75%/68%. The overall PPV/NPV (ASC-US+) for SP and TP were 55%/95% and 34%/93%, respectively. For LSIL+, SP sensitivity was 90% and TP sensivity was 62%. Sensitivity of both specimen types was 100% for HSIL+.

Conclusions: Sensitivity for abnormal SP slides was at or above the level of manual glass slide screening. Specificity, as expected, was lower using remote low resolution static images as observers tend to overcall. The telecytology method performs in a manner that would be acceptable as an initial triage to further on-site glass or remote whole slide image review (for SP 74% of cases were correctly classified and only 3% of cases would have been triaged erroneously to a patient's detriment). TP may not be optimal as the lower cell density on the TP presents fewer cells in each FOV, hence decreasing overall sensitivity; and the FocalPoint device has been optimized for its primary clinical application of screening SP slides.

365 BRAF Mutation (V600E) in Papillary Carcinoma Identified on LBC-Processed Thyroid Aspiration Biopsies.

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Background: Activating mutations of the BRAF gene are observed in a proportion of papillary thyroid carcinoma (PTC). The most common mutations involves the V600E locus and has been identified in 70% of the classic variant of PTC and more than 80% of the tall cell variant. But, it has not been detected in benign lesions and in the majority (80%) of the follicular variant of PTC. The identification of BRAF V600E mutation in a PTC diagnosed on liquid based cytology (LBC) may be useful to predict the prognostic outcome of PTC and to direct the clinical management of patients. To determine the utility of the mutational analysis in thyroid fine needle aspiration (FNA),10 cases with histology were examined.

Design: Ten cases of thyroid carcinomas on LBC-FNA,underwent BRAF mutational analysis. The needle with the material was rinsed in a haemolytic-preservative solution Cytolit (Hologic Co). The cells were spun at 1500 rpm (rotations per minute) then the sediment transferred in the PreservCyt (Hologic Co) solution to be processed with the T2000 automated processor according to the manufacturer's recommendations. The resulting slide was fixed in 95%ethanol and stained with Papanicolau. DNA extraction was performed on FNA sample ThinPrep 2000 using the QIAamp tissue kit (Qiagen). After PCR amplification the fragment spanning the Braf exon 11 and 15 were treated with EXOSap (UBS, Sial) and directly sequenced using BigDye Terminator kit v3.1 (Applied Biosystem) using the same primers of the PCR amplification, in an ABI PRISM 3100 Genetic Analyzer (Applied Biosystems).

Results: Six cases (60%) showing a BRAF gene mutation were identified in LBC-FNA. Among them, 3 were histologically multifocal PTC with nodal metastases, 1intraglandular microPTC, 2tall cell variants of PTC multifocal with positive nodes. The remaining 4 cases without BRAF mutation were 2follicular variants ,1columnar cell carcinoma and 1PTC.

Conclusions: The V600E BRAF mutation can be successfully identified on LBC material even a few months after the FNA. This parameter may predict the clinical behavior of cases diagnosed as PTC on FNA and allowing a correct surgical strategy without repeating the biopsy.

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366 Spectral Cytopathology of Cervical Cells Infected with Human Papillomavirus.

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Background: Infrared micro-spectral imaging (IRMSI) is a novel approach for cytological diagnosis. This optical technique detects subtle biochemical changes in cells. Computer based algorithms analyze spectral differences within individual cells and provides an objective and reproducible tool for cytological diagnosis. These spectral differences may be observed before morphological changes are seen, providing additional sensitivity for earlier diagnosis. We report the use of this technique to analyze both healthy and human Papillomavirus (HPV) infected cervical squamous cells.

Design: Spectral Cytopathology (SCP), a combined technique employing IRMSI for data collection and analysis by multivariate statistics, was used to investigate exfoliated cervical samples and cultured cervical cells (C33A, HeLa, CaSki, SiHa) with different numbers of episomal copies of HPV. All specimens were prepared onto infrared (IR) microscope slides. The unstained slides were interrogated by a beam of IR light that samples pixels (6.25 µm x 6.25 µm in size) on the sample spot. A 4 mm x 4 mm IR image, which contains 409,600 complete IR spectra, was recorded from each sample. Pixels located on an individual cell were co-added, resulting in one IR spectrum for each cell that describe its discrete biochemical composition. Approximately 500-1000 cells were collected from each sample spot. Multivariate methods of analysis were then used to differentiate the cellular spectra and correlate them with conventional cytology & HPV DNA testing.

Results: This study included 48 clinical samples that were diagnosed by traditional cytology, assayed for high-risk strains of HPV (Digene Hybrid Capture II), and analyzed by SCP. The study reports a sensitivity of 91% and a specificity of 57% to detect high-risk HPV. We believe our reported poor specificity could be due to: the reported low specificity of the Digene test when compared to more sophisticated PCR based methods of viral detection, or SCP is detecting the presence of low-risk strains of HPV which are not detected by the current DNA test. We are conducting a study on cultured cervical cells that compose a different number of episomal copies of high-risk strains of HPV to determine SCP's sensitivity for viral load and latent viruses. These results will be reported.

Conclusions: We have identified biochemical differences in morphologically normal cells that contain high-risk strains of HPV by use of SCP. These results suggest that SCP could provide a potent adjunct diagnostic tool for cytopathologists.

367 Dual CK5/P63 Staining in Pulmonary Squamous Cell Carcinoma: Utility in Cell Blocks.

N Fatima, C Cohen, MT Siddiqui. Emory University Hospital, Atlanta, GA.

Background: Squamous cell carcinoma (SQCC) is the second most common type of lung cancer, constituting about 30% of lung cancers. Increasing demand for accurate differentiation of SQCC from other subtypes can be challenging for pathologists. This is more so in fine needle aspirations (FNA) since SQCC may show degenerative changes which can be perceived as cytoplasmic vacuoles with abortive lumen formation. Immunohistochemistry (IHC) is a valuable adjunct in the sub-classification of non small cell lung carcinoma. Stains used for this purpose include TTF-1, CK7, CK20, P63 and CK5/6, with, immunoreactivity for p63 and/or CK5/6 basically restricted to SQCC. The combination of these two stains has been reported to have a high reliability for diagnosing SQCC of the lung.

Design: To evaluate the utility of this dual stain, we studied FNA cell blocks (CB) of 24 SQCC and 34 adenocarcinoma (ADC) of the lung. Dual CK5/P63 IHC was performed. Red CK5 cytoplasmic stain and brown P63 nuclear stain was examined. For positive control, a histologic section of a primary lung SQCC was used.

Results:

Table 1:

	SQCC	ADC
Dual CK5/P63	17/24 (70%)	0/34 (0%)
P63 Component Alone	2/24 (8%)	3/34 (8%)
CK5 Component Alone	2/24 (8%)	0/34 (0%)

Table 2

	Sensitivity	Specificity	Accuracy	PPV
Dual CK5/P63 Stain in SQCC	70%	100%	88%	100%

Conclusions: Cytologic evaluation with dual staining for CK5/P63 has a high sensitivity and specificity for diagnosing lung SQCC. This dual stain can distinguish SQCC from ADC with an accuracy of 88% and a positive predictive value of 100%, which is extremely critical for diagnostic and therapeutic decisions.

368 TTF-1 and Napsin-A Double Staining in Lung Adenocarcinoma: Diagnostic Utility in Fine Needle Aspirations.

N Fatima, C Cohen, MT Siddiqui. Emory University Hospital, Atlanta, GA.

Background: Lung cancer is the most common type of cancer in terms of mortality worldwide. Recent FDA approval of Avastin in the treatment of non-squamous cell lung cancer and its contraindication in squamous cell carcinoma (SQCC) has made it crucial to accurately diagnose the different types of non-small cell lung cancers. Immunohistochemistry (IHC) for thyroid transcription factor-1 (TTF-1) is widely used in the diagnosis of lung adenocarcinomas (ADC). It is positive in approximately 75% of lung ADC and negative in most SQCC and ADC of other organs. A new promising marker, Napsin-A, has been detected in the cytoplasm of type 2 pneumocytes and alveolar macrophages. It is an aspartic proteinase involved in the processing of surfactant protein B and is strongly positive in up to 80% of primary lung ADC by IHC. Small cell carcinomas and SQCC of the lung have been shown to be negative for Napsin-A. A combination (Double stain) of these two stains (TTF-1 and Napsin-A) has been proposed to achieve higher sensitivity and specificity.

Design: FNA cell blocks of 36 lung ADC and 26 lung SQCC were studied. IHC was performed on formalin-fixed paraffin-embedded cell blocks. Expression of Napsin-A as cytoplasmic red stain and TTF-1 as nuclear brown stain were identified easily. For positive control, lung ADC was used.

Results:

Table 1:

	TTF-1/Napsin-A Double stain	TTF-1 Alone	Napsin-A Alone
ADC	27/36 (75%)	4/36 (11%)	4/36 (11%)
SQCC	3/26 (11%)	6/26 (23%)	0/26 (0%)

Table 2:

	Sensitivity	Specificity	PPV	NPV	Accuracy
TTF-1/Napsin-A Double Stain	75%	88%	90%	71%	80%

Conclusions: Double staining for TTF-1 and Napsin-A gives a sensitivity of 75% and specificity of 88% for ADC. An additional 8 ADC stained for either TTF-1 or Napsin-A. Utilizing this dual stain, lung ADC is diagnosed with an accuracy of 80%. We conclude that TTF-1/Napsin-A double stain is a better diagnostic technique than TTF-1 or Napsin-A alone, and helps in diagnosis of ADC with a positive predictive value of 90% and negative predictive value of 71%. Only 11% of SQCC stained with the double stain, an additional 23% with TTF-1 alone, but none for Napsin-A alone.

369 Grading Dysplasia in Mucin-Producing Cystic Neoplasms of the Pancreas on Fine Needle Aspiration.

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Background: In mucin-producing neoplasms of the pancreas, the best predictor of prognosis is the presence of invasive carcinoma. 10% of mucinous cystic neoplasms (MCN) and 30% of intraductal papillary mucinous neoplasms (IPMNs) harbor invasion. Fine needle aspiration (FNA) is a primary modality for diagnosis of these lesions; however, grading dysplasia and detecting invasion in these lesions by cytology poses a diagnostic challenge. Our aim is to determine cytomorphologic features of high-grade dysplasia and invasion.

Design: A database search was performed for purely cystic, pancreatic FNAs over a 10 year period (2000-2010) which had histologic follow-up. Non-diagnostic cases on FNA were excluded. Diff-Quik and Papanicolau stained direct smears were scored blindly for the presence of 23 cytologic features without knowledge of histologic outcome: Mucinous background, cellularity, small cell groups, flat sheets, cellular crowding, 3-dimensional (3D) clusters, glandular formation, necrosis, nuclear pleomorphism, high N/C ratio, nuclear grooves, nuclear membrane irregularity, nuclear overlapping, nuclear molding, nucleoli, nuclear enlargement, parachromatin clearing, hyperchromasia, single intact cells, papillae, mitoses, and atypical mitoses. On a four point scale, scores of 0 or 1 were considered negative for a feature, while scores of 2 or 3 were considered positive. Chi-square analysis was performed to determine the cytomorphologic features that are predictive of dysplasia.

Results: 31 cases of MCNs by FNA (24 female,7 male, average age = 64 years), of which 12 were adenocarcinomas, 13 were IPMNs [6 low, 4 moderate, 3 high-grade dysplasia] and 6 were mucinous cystadenomas [4 low, 1 moderate, 1 high-grade dysplasia] on histology. The cytomorphologic features most predictive of high-grade dysplasia and carcinoma combined were: Presence of prominent nucleoli (P=0.031), small cell groups (P=0.006), 3D clusters (P=0.027). In addition, the presence of single intact cells (P=0.022) and mitoses (P=0.038) were found to be suggestive of invasion.

Conclusions: - The diagnosis of high-grade dysplasia and invasive carcinoma in mucin-producing cystic neoplasms of the pancreas can be suggested using the cytomorphologic features.

- High-grade dysplasia and invasive carcinoma was found in a significant number [55%] of the cases presented in this study

- Grading of dysplasia on FNA may aid in guiding theraputic intervention in select patients

370 Arginase-1 Is a More Sensitive Marker of Hepatic Differentiation Than HepPar-1 in Fine Needle Aspiration Biopsy Specimens.

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Background: Distinguishing hepatocellular carcinoma (HCC) from adenocarcinoma in fine needle aspiration biopsies (FNAB) is often diagnostically challenging. Arginase-1 was recently described as a marker of hepatic differentiation in surgical resection specimens. We compared the reactivities of Arginase-1 and HepPar-1 in FNAB of HCC and adenocarcinoma involving the liver.

Design: Seventy-four FNAB including 31 primary or metastatic HCC (27 well/moderately differentiated and 4 poorly differentiated) and 43 adenocarcinomas involving the liver were evaluated. Immunohistochemical staining for Arginase-1 (polyclonal, Sigma-Aldrich, 1:200) and HepPar-1 (clone OCH1E5, Dako, 1:200) was performed on formalin-fixed cell block material. Only cytoplasmic staining was scored as positive staining for both Arginase-1 and HepPar-1. Staining intensity (weak versus strong) and extent (focal, <10% versus diffuse, >10%) were scored.

Results: Arginase-1 was more sensitive (27/31, 87%) than HepPar-1 (25/31, 81%) for HCC (Table 1). Arginase-1 more often demonstrated strong (26/27, 96%) and diffuse (18/27, 67%) staining in HCC compared with HepPar-1 (20/25, 80% and 14/25, 56%, respectively). Arginase-1 staining was identified in adenocarcinomas of the pancreas, breast, and colorectal origin, although reactivity was typically weak and focal. HepPar-1 demonstrated strong and diffuse reactivity in cases of gastric, pulmonary, and prostatic adenocarcinoma.

Arginase-1 and HepPar-1 in Hepatocellular Carcinoma and Adenocarcinoma in FNAB

Tumor Type	Arginase-1 Positive (%)	HepPar-1 Positive (%)
Well/moderately differentiated HCC	25/27 (93)	24/27 (89)
Poorly Differentiated HCC	2/4 (50)	1/4 (25)
Colorectal and Anal carcinoma	2/16 (13)	0/16 (0)
Breast adenocarcinoma	1/12 (8)	0/12 (0)
Pancreatic adenocarcinoma	3/7 (43)	0/7 (0)
Pulmonary adenocarcinoma	0/3 (0)	1/3 (33)
Prostatic adenocarcinoma	0/2 (0)	1/2 (50)
Gastric adenocarcinoma	0/1 (0)	1/1 (100)
Intrahepatic cholangiocarcinoma	0/1 (0)	0/1 (0)
Uterine carcinoma	0/1 (0)	0/1 (0)

Conclusions: Arginase-1 is a more sensitive marker of hepatic differentiation than HepPar-1 in FNAB specimens. In addition, Arginase-1 exhibits stronger and more diffuse positive staining in HCC than HepPar-1 making interpretation easier in limited FNAB samples. Arginase-1 is not entirely specific for hepatic differentiation, as focal, weak immunoreactivity can be identified adenocarcinomas of pancreatic, breast, and colorectal origin.

371 Impact of HPV Vaccination on HPV Genotypes in Women with Atypical Cells of Undetermined Significance (ASC-US) in a Low-Risk Screening Population.

RG Gamez, SE Pambuccian, B Thyagarajan, SM Cook, S Amirouche, EH Gulbahce. University of Minnesota, Minneapolis.

Background: HPV vaccination with the quadrivalent vaccine Gardasil® was approved by the FDA in June 2006 for use in females aged 9 to 26 and may also be useful in women aged 27-45. To date 44.3% of all U.S. females aged 13-17 have recieved at least one dose (MMWR 2010;59:1018-23). The aim of this study was to determine the potential impact of HPV vaccination on the HPV genotypes detected in women with ASC-US.

Design: All liquid-based (Surepath®) Pap tests diagnosed as ASC-US from 12/3/2002 to 6/30/2009 who had concomitant HPV genotyping were identified. Pap tests were diagnosed according to the 2001 Bethesda System; HPV tests were performed by a "home-brew" PCR-based method using the MY9/11 L1 consensus primers. The 13 HPV types included in the hc2 cocktail (16,18,31,33,35,39,45,51,52,56,58,59,68) were considered as high-rik HPV (HR-HPV). During the study period there were no major changes our screening population, laboratory personnel or molecular methods used. The study interval was divided into two periods: "pre-vaccination" (12/3/2002-6/30/2006) and "post-vaccination" (7/1/2006-6/30/2009). The frequecies of HPV 6, 11, 16 and 18 were compared between the two periods using χ^2.

Results: During the study period our laboratory processed 391,845 Pap tests.

	Total Pap tests	% ASC-US	ASC-US Age (mean± SD)	ASC/SIL ratio
Pre-vaccination period	215,784	5.31%	35.14 ± 12.89	2.48
Post-vaccination period	176,061	4.96%	35.62 ± 13.16	1.88
Total	391,845	5.15%	P val .018	2.18

16594 cases of ASC-US from women aged 11 to 89 (mean, 35.37 ± 13.02 SD) with concomitant valid HPV genotyping results were identified.

	Number ASC-US	any HPV type	hr-HPV+	HPV6	HPV11	HPV16	HPV18
Pre-Vaccine	8636	3767 (35.3%)	1773(16.65)	202 (6.3%)	24 (0.7%)	634 (19.7%)	155 (4.8%)
Post-Vaccine	7958	3284 (42.0%)	1666 (20.9%)	171 (5.5%)	28(0.9%)	522(17.7%)	169 (5.4%)
p value		<.001	<.001	.182	.578	.039	.305

There was an increase in the total HPV+ and HR-HPV+ rate in the post-vaccination period, most likely due to the more restrictive criteria for the diagnosis of ASC-US in this period, reflected in the lower ASC-US and ASC/SIL ratios. Despite this increase in the frequency of overall HPV+ and HR-HPV+ cases, there was a significant reduction in HPV16+ ASC-US cases.

Conclusions: Our study shows that, despite the relatively low vaccination rate, the incomplete vaccination status and the targeting of only younger women, HPV vaccination has resulted in a decrease of HPV16 as a cause of ASC-US in our population.

372 Impact of HPV Vaccination on HPV Genotypes and Follow-Up Biopsy Results of Women >30 with Normal Pap Test Results.

RG Gamez, A Samad, L Xie, S Amirouche, EH Gulbahce, SE Pambuccian. University of Minnesota, Minneapolis.

Background: Gardasil®, a trivalent HPV vaccine that confers protection against infections with HPV6,11,16 and 18 has been used since its FDA approval in June 2006. Despite the fact that it has been approved for use in females aged 9-26, it has also been sporadically used in women over 27.

Women >30 are frequently cotested with Pap tests (PT) and HPV tests, a strategy that minimizes the likelihood of missing significant cervical squamous precursor lesions (CIN2/3), but results in the detection of very few additional CIN2/3 lesions in women

with PT interpreted as normal (NILM) in high-quality cytology laboratories.
The aim of this study was to determine the potential impact of HPV vaccination on the HPV genotypes and follow-up CIN2/3 biopsies of women with normal PT.
Design: All liquid-based (Surepath) PT interpreted as NILM from women >30 that had concomitant HPV genotyping performed from 01/01/2001 to 06/30/2009 were identified. HPV testing was performed by a "home-brew" PCR-based method using MY01/11 primers.The study period was divided into a "pre-vaccine" period (P1)(1/1/01 to 6/30/06) and a "post-vaccine" period (P2)(7/1/06-6/30/09) and differences in the frequency of all HPV types identified, high-risk (hr)-HPV types identified and HPV6, 11, 16 and 18 as well as in the follow-up rate of CIN2/3 detected within 6 months of the PT were determined using $\chi 2$.
Results: 9498 women >30 with normal PT results had HPV genotyping performed during the study period, 2312 (mean age of 45.14±11.53) in P1 and 7186 women (mean age 44.9±10.7) in P2.

	Total Number	Any HPV Type	hr-HPV Type	HPV6	HPV11	HPV16	HPV18
"Pre-vaccine" period	2312	163 (7.1%)	64 (2.7%)	10 (0.4%)	2 (0.1%)	38 (1.6%)	4 (0.2%)
"Post-vaccine" period	7186	533 (7.4%)	138 (1.9%)	24 (0.3%)	6 (0.1%)	64 (0.9%)	12 (0.2%)
p value		.5	.01	.5	.6	.003	1

There was a significant decline in all hr-HPV types and in the HPV16 rate and combined rate of all HPV types targeted by the vaccine (6/11/16/18)(P<0.001) in the P2.
275 women were biopsied, of which only 9 received a diagnosis of CIN2/3., 6 (0.26%) in P1 and 3 (0.04%) in P2 (p=0.0087).
Conclusions: We observed a significant decline in the rate of hr-HPV types and HPV16 and the combined rates of HPV6/11/16/18 in women with normal PT results in the post-vaccine period. This was associated with a decline in the already low CIN2/3 rate in these women. Since women >30 are not included in the intended HPV vaccination target population, these declines may represent a "herd effect".

373 Utilization of p16 and ProEx C Staining in Differential Diagnosis of Atypical Cells in Liquid-Based Pap Tests during Menstruation.

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Background: Though the Pap test is an effective screening tool for cervical carcinoma, some diagnostic pitfalls do exist. This is especially true when evaluating hyperchromatic crowded groups (HCGs) in Pap tests from women during menstruation due to similar morphological appearances between endometrial clusters and precancerous or malignant HCGs. Previous studies demonstrated that p16 and ProEx C are often expressed in cervical precancerous or malignant lesions. We studied the results of p16 and ProEx C on cell blocks from Pap tests with atypical cells during menstruation to attempt to differentiate endometrial cells from cervical dysplastic lesions.
Design: Immunohistochemical stains for p16 and ProEx C were performed on 21 cell blocks prepared from residual liquid-based cervical material with endometrial contamination. The cases include 10 benign (NILM), 4 low grade squamous intraepithelial lesion (LSIL), 5 high grade squamous intraepithelial lesion (HSIL), 1 endometrial adenocarcinoma, and 1 metastatic breast carcinoma. Endometrial biopsies from non-neoplastic cycling endometrium were studied as a control.
Results: Strong, diffuse and full thickness staining pattern for p16 and ProEx C was observed in all cases of HSIL. The cases of LSIL were negative for ProExC with weak patchy basal-predominant staining for p16. In contrast, all cases of NILM were negative for both p16 and ProEx C stains. The endometrial cells in the background were either negative or had focal patchy staining. Weak staining was focally seen in 1 case of endometrial adenocarcinoma and 1 case of metastatic breast carcinoma. Endometrial biopsies at various functional phases showed patchy staining pattern for both p16 and ProEx C.
Conclusions: ProEx C and p16 are strongly and diffusely positive in HSIL. ProEx C is negative in LSIL with p16 only focally positive in the basal layer. The stains should be interpreted with caution because patchy/mosaic staining pattern can be seen in benign endometrial tissue, especially in proliferative phase or with tubal metaplasia. In addition, ProEx C staining is usually cleaner than p16 staining. Therefore, combination of both p16 and ProEx C immunostains and being aware of various staining patterns are critical in accurate interpretation of results. The authors believe the proposed method will be significantly helpful in differential diagnosis of HCGs in menstrual Pap specimens.

374 The Utility of TTF-1 & Napsin A Dual Immunostain in the Distinction of Metastatic Lung Adenocarcinoma from Other Metastatic Adenocarcinoma in Body Fluid Specimens.

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Background: Discrimination of metastatic adenocarcinoma of pulmary origin from metastatic adenocarcinoma of other primary sites is challenging. TTF-1 has been used as the predominant immunomarker to confirm lung origin. Napsin A is a recently charaterized marker which has shown high specificity for lung tissue in surgical pathology specimens. In this study we evaluated whether novel dual TTF-1 & Napsin A immunostain increases the sensitivity and specificity in diagnosing metastatic lung adenocarcinoma in body fluids.
Design: Paraffin embedded adequately cellular body fluid cell blocks from 19 consecutive patients with a diagnosis of metastatic adenocarcinoma were retrieved (12 metastatic lung adenocarcinoma, 2 colon, 5 breast). Adequately cellular levels were immunostained using novel TTF-1 & Napsin A multiplex cocktail. The presence of one or more individual cells with convincing brown nuclear TTF-1, red cytoplasmic Napsin A staining, or cells with TTF-1/Napsin A co-expression were interpreted as "positive".
Results: Of the 12 metastatic lung adenocarcinoma, 11 were positive for both TTF-1 & Napsin A; 1 case was negative for TTF-1 but was positive for Naspin A, and 1 case was negative for Napsin A but was positive for TTF-1. The 2 colon and 5 breast cases were all negative for both TTF-1 and Napsin A (specificity 100% and sensitivity 100%).
Conclusions: The dual immunostain TTF-1 & Napsin A is highly specific and sensitive for lung adenocarcinoma, and can be highly useful in differentiating metastatic lung adenocarcinoma from metastasis from othe body sites including breast and colon.

375 HER-2/Neu Status Can Be Reliably Determined in Cytologic Breast Cancer Specimens Using Bright-Field Microscopy and Silver In-Situ Hybridization (SISH).

Z Ghorab, RS Saad, S Noy, WM Hanna, S Nofech-Mozes. Sunnybrook Health Sciences Centre, Toronto, ON, Canada.
Background: Accurate HER-2 testing of all breast cancer patients at primary diagnosis is essential for optimal disease management. ASCO/CAP guideline recommendations for HER-2 testing emphasize the need for standardization of preanalytical variables, especially formalin fixation. Accordingly, any change in testing protocol should be validated. Alcohol fixation may alter immunoreactivity; however there is only limited data on reliability of in situ hybridization in alcohol fixed cytologic specimen (CS). This study was designed to validate SISH in alcohol fixed cell blocks.
Design: We identified 50 pairs of CS positive for malignant cells and a corresponding breast cancer surgical specimen. This set of cases was specifically enriched in HER-2 positive cases. The gold standard for validation was HER-2 status that was determined on the FFPE surgical specimens by IHC. Equivocal IHC cases were further tested by FISH or SISH. CS were fixed in alcohol based preparation: 48 Saccomanno Collection Fluid and 2 in Cytolyt. HER-2 gene amplification status was determined on cell blocks using the Ventana Inform HER-2 SISH kit (Tucson, Arizona). Amplification was determined when the SISH HER-2 /CEP17 ratio was greater than 2.2. Two pathologists reviewed the CS and determined the SISH ratio and quantify malignant cells in a 3 tiered system (cellularity:1= 1-20 cells, 2=21-60 cells, 3=>60 cells). The concordance between HER-2 testing by SISH on CS was compared to that of FFPE surgical specimens.
Results: There were 42 cases with 3+ cellularity, 5 cases with 2+ and only 2 cases with 1+ cellularity (both were amplified). SISH reaction was successful in 49/50 (98%) cases. In one case, the test was uninterpretable due to poor HER-2 signal. All 22 Her2-positive surgical cases were CS-amplified and all 27 Her2-negative surgical cases were CS-not amplified. Overall there was a 100% concordance between Her2 status determined on alcohol fixed CS by SISH when compared with FFPE surgical specimens.
Conclusions: SISH reaction was successful in 98% of the cases. Our study validated SISH as a reliable accurate test for Her2 on alcohol fixed cytologic specimens. SISH allows for bright field microscopy which is advantageous over dark field traditional florescence technique, in particular in CS even with low cellularity.

376 Cyanophilic Small Atypical Parakeratotic Cells in ThinPrep Cervical Cytology: A Pitfall Leading to HSIL or ASC-H Misinterpretation.

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Background: We previously reported significance of small atypical parakeratotic (SAPK) cells in liquid-based cytology (SurePath™) smears. A subset of SAPK cells may be misinterpreted as HSIL or as "Atypical squamous cells, rule out high grade squamous intraepithleial lesion (ASC-H)". However, follow-up biopsies reveal frequent association with negative results or cytopathic effect of Human Papilloma Virus (HPV) without dysplasia. The goal of this study is to evaluate SAPK cells [both orangeophilic (SAPK-O) and cyanophilic (SAPK-C)] in ThinPrep™ smears (TP) with follow-up biopsy specimens.
Design: Out of 89138 TP (during the year 2009), 45 ASC-H and 242 HSIL cases with availability of follow-up biopsies were included.
TP were studied for association with SAPK cells. SAPK cells showed: a. Relatively cohesive hyperchromatic crowded groups of small parakeratotic cells with atypical nuclei (vs non-cohesive checker board pattern in CIN2); b. Recognizable intercellular cytoplasmic borders (vs syncitial pattern in CIN3); c. Cyanophilic cytoplasm with slightly angulated and sharp straight peripheral margins (with variable focal acido-orangeophilia); d. High N/C ratio with ill-defined spaces around nuclei reminiscent of koilocyte.
Results: The correlation of biopsy results with cytopathologic findings is shown in table 1. Out of 16 SAPK with or without ASC-H, 9 revealed SAPK-C, 4 SAPK-O, and 3-both SAPK-O and SAPK-C.

Correlation of cytopathology findings with biopsy results

Initial Cytopathology	Review findings	Bx Negative	Bx HPV	Bx-CIN1	CIN2 & above
ASC-H (54)	ASC-H cells only (38)	21 (38%)	1 (2%)	8 (15%)	8 (15%)
	ASC-H cells with SAPK (10)	3 (6%)	0 (0%)	0 (0%)	7 (13%)
	SAPK only (6)	4 (7%)	1 (2%)	1 (2%)	0 (0%)
HSIL (218)	HSIL cells only (159)	26 (12%)	0 (0%)	21 (10%0	112 (51%)
	HSIL cells with SAPK (59)	26 (12%)	1 (0.5%)	2 (1%)	30 (13.5%)
	SAPK only (0)	0	0	0	0

Bx, Biopsy; SAPK, small atypical parakeratotic cells

Conclusions: Study demonstrated that SAPK cells are also pitfall in TP with tendency for ASC-H and even HSIL interpretation. This is more often with completely cyanophilic SAPK cells. Familiarity with the cytomorphologic spectrum of SAPK cells may prevent their potential misinterpretation as HSIL or ASC-H cells.

377 The Use of Telecytopathology (TeleCyP) in Real Time On-Site Evaluation of Fine Needle Aspiration Specimens.

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Background: Telepathology can be challenging to implement as a diagnostic modality in the field of cytopathology; because precise cytopathologic interpretation requires examination of the finest cellular details. Numerous studies have demonstrated that both static and dynamic TeleCyP are useful in providing diagnostic services at remote sites by assessing specimen adequacy, thereby allowing a more effective use of cytopathologist's time. In this study, we have evaluated whether real-time TeleCyP can prove advantageous for on-site assessment in a busy fine needle aspiration (FNA) practice such as at our institution.

Design: A cohort of already diagnosed 38 pancreatic FNAs performed by endoscopic-ultrasound (EUS) was selected to validate the effectiveness of TeleCyP in providing on-site adequacy assessment and interpretation. A cytopathology fellow was responsible for driving the Diff-Quik® stained slides (1 to 2 per case) on the microscope equipped with triple chip high-speed digital video camera (Optronics, Coleta, California) and selecting the fields of interest. Two cytopathologists who were blinded to the final cytopathologic diagnosis, independently examined the images on a personal computer employing "Axis Video Management Software" (Lund, Sweden) in real time. At the same time the cytopathologist was in constant communication with the cytopathology fellow to inquire regarding the pertinent clinical details of the case and pinpoint the fields/cells that needed to be examined on high power.

Results: The average time spent for the TeleCyP on-site diagnosis was 49.2 seconds (range 15 to 165 seconds). An average of 1.6 medium-power fields (10X and 20X) and 1.5 high-power fields (40X) were examined; average time spent in low power field (5X) scanning was 28.4 seconds. There was complete agreement between the TeleCyP on-site and final cytopathology diagnoses in 38 cases (16 adenocarcinomas, 7 mucinous cystic neoplasms, 6 neuroendocrine neoplasms, 5 non-neoplastic conditions and 1 solid pseudopapillary tumor). The discrepancy was noted in only 3(3/38 8%) benign cases which were interpreted as tumor/suspicious for tumor on TeleCyP.

Conclusions: This validation study shows that real-time TeleCyP can be successfully employed for on-site evaluation in a busy FNA service setting. Evaluation of additional cases from other organ systems will be required before we incorporate the routine use of TeleCyP in our practice.

378 Multispectral Imaging as an Adjunct for Classification of Thyroid Fine Needle Aspirates.

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Background: Fine-needle aspiration (FNA) biopsy is the standard method for evaluation of thyroid nodules. The Bethesda System improves the reliability of FNA diagnosis through standardization of diagnostic features, but equivocal cases persist. Multispectral imaging of FNA biopsies captures visible light information beyond the capacity of the human eye and could increase the accuracy of FNA biopsy in classifying challenging cases. In this study, we focus on the distinction between papillary carcinoma (PTC) and goiter, and the distinction between follicular carcinoma (FC) and follicular adenoma (FA).

Design: 84 archived cytology cases utilizing the Bethesda System from 2007-2009 were collected. A CRI Nuance multispectral camera and the accompanying software were used to image the thyroid FNAs and develop computer-based classification algorithms (classifiers). We developed two classifiers. The first, "PTC/G," classifies FNA images as either PTC or goiter. The second, "FC/FA," classifies FNA images as either FC or FA. Classifiers segregate images into regions based on represented wavelengths and then compute areas of the regions; in the case of PTC/G, this corresponds to regions of PTC, goiter, or neither feature. If the ratio of PTC area to goiter area is over a threshold value, the image as a whole is classified as PTC; FC/FA functions similarly. PTC/G was developed using 40 images of PTCs and goiters and tested on a distinct set of 30 PTC and 30 goiter images. FC/FA was developed using 35 images taken from surgically confirmed FC and FA cases and tested on a distinct set of 14 FC and 15 FA images.

Results: A Receiver Operating Characteristic (ROC) curve was generated for the PTC/G classifier with an Area Under the Curve (AUC) of 0.90. The AUC of the FC/FA classifier ROC curve was 0.76. Choosing threshold ratios of malignant to benign areas for each classifier, we generated three specific tests which are summarized in the table.

Summary of tests

Test	Sensitivity	Specificity
PTC/G screen	0.93	0.73
PTC/G diagnostic	0.70	0.90
FC/FA preliminary	0.73	0.64

Conclusions: We demonstrate the feasibility of using multispectral imaging as an adjunct to the Bethesda FNA classification system. The PTC/G classifier was able to accurately distinguish between PTC and goiter with sensitivity and specificity rivaling current cytology standards. The FC/FA classifier showed preliminary ability to distinguish between FC and FA. Future work will improve the accuracy of both classifiers, focusing on cases in which cytologic diagnosis is uncertain.

379 The Value of a Repeat Cytology at the Time of First Colposcopy: A Retrospective Analysis of 1087 Cases.

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Background: After a referral for an "abnormal" Pap test interpretation, performing a repeat Pap test at the time of colposcopy is not an uncommon practice, although the validity and clinical usefulness of this practice is unclear. In this large scale retrospective study, we assess the value of repeat cytology in this context.

Design: We reviewed the records for 1087 consecutive patients with abnormal Pap tests (i.e. interpretations of ASC-US and above: "referral cytology") that was followed (within 12 months of the referral) by a colposcopic examination in which both a Pap test ("repeat cytology") and histological evaluation (i.e. biopsy or ECC) were performed. Repeat cytology was considered "clinically useful" if the results could conceivably have impacted the decision to proceed with or defer a more invasive diagnostic/therapeutic evaluation, such as the performance of a loop electrosurgical excision procedure (LEEP), based on current ASCCP guidelines (e.g LSIL referral cytology followed by HSIL repeat cytology and a CIN 1/negative biopsy, a scenario that may prompt consideration of a LEEP. All cases were further categorized into risk groups: "Low risk (LR)" cytology included the following diagnostic categories: NILM, ASC-US, and LSIL; "Intermediate risk" included LSIL-H and ASC-H, and "High risk (HR)" included HSIL, atypical glandular cells, and carcinoma.

Results: The overall agreement between referral and repeat cytology was 86%, in that 86% of all cases were in the same risk category in the referral and repeat cytologies. After excluding cases with "intermediate risk" referral or repeat cytology, we found that referral LR/repeat HR combination was seen in 49 (4.9%) of the 996 cases, whereas HR/LR and HR/HR combinations were seen in 3% and 3% of the 996 cases respectively. The sensitivity, specificity, positive predictive value, and negative predictive value on the ability of repeat cytology to detect the most clinically significant lesion (using findings from the concurrently obtained biopsy as gold standard) were 64%, 97%, 76% and 95% respectively. Overall, repeat cytology provided potentially clinically useful information in only 36 (3.6%) of the 996 cases, including 41% and 1.8% of the HSIL and LSIL referral cytology cases respectively.

Conclusions: Repeat cytology at the time of first colposcopy provides potentially clinically useful information in only a small percentage of cases overall, but in a substantial proportion of HSIL referral cytology cases, justifying its continued performance at least in this subset of patients.

380 Correlation of Expression of HER2 in Circulating Tumor Cells and in Corresponding Primary Tumors in Breast Cancer Patients.

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Background: Enumeration of circulating tumor cells (CTCs) is a rapidly growing new diagnostic test to help manage oncology patients. The prognostic importance of monitoring CTCs levels for recurrence and response to chemotherapy in breast cancers has been shown by prior studies. These assays also have the potential benefit to guide treatment decisions based on the molecular profile of the tumor before therapy. A positive HER2 status is associated with aggressive tumor behavior. We propose to determine the differential protein expression of HER-2 in the primary tumors and CTCs.

Design: Whole blood was drawn from patients with metastatic breast carcinoma (n=323) The CellSearch System (Veridex,LLC,Warren,NJ) the only FDA approved technology was used to analyse CTCs which consists of a semiautomated sample preparation system and the CellSearch Epithelial Cell kit to immunomagnetically enrich cells expressing epithelial cell adhesion molecule. Circulating tumor cells are defined as intact tumor cells that express the epithelial cell marker (CK-PE) with a nucleus that stains positive for the nucleic acid dye (Dapi) and are negative for the leukocyte marker CD45. CTC analysis were done by cytotechnologists and pathologists certified by Veridex.

Results: CTCs were detected in 44% of metastatic breast cancer patients with frequency range of 1-701, mean 7.1, SD=44. CTCs were investigated for HER2/neu in 20 cases. HER2 -positive CTCS were present in 16 cases with a frequency range of 1-224, mean 2.5, SD=44. HER2 expression of primary tumors as assessed by clinical immunohistochemistry were compared to CTCs HER2 expression.

Comparison of HER2 expression in primary tumor and CTCs

	Primary Tumor	CTC
HER2 +	14	16
HER2 -	2	0

The HER2 status of the metastases when available was reported to be similar to that of the primary tumor. All HER2 reported as 1+ or 2 + in surgical samples were not amplified by FISH.The HER2 expression of primary tumors matched that of the CTCs in 14 of 16 cases. In two cases the HER2 of the primary tumor was negative with HER2 -positive CTCs.

Conclusions: The CTC phenotype may accurately reflect the tumor phenotype than expression in the primary or metastatic sites. Immunohistochemical (IHC) evaluation of HER-2 protein by chromogenic IHC is not only subjective and semiquantitative, but has intrinsic variables. We hypothesize that the HER-2 expression in CTCs may be true reflection of metastatic clone derived from the primary tumo and may predict response to targeted therapy despite the negative HER2 expression in the primary tumor.

381 Significance of Granulomas Detected in Mediastinal Lymph Nodes by Fine Needle Aspiration (EUS-FNA and EBUS-TBNA) in Patients with Malignancies.

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Background: EUS-FNA and EBUS-TBNA are minimally invasive biopsy techniques useful in the diagnosis of mediastinal lymph node metastases from intrathoracic and extrathoracic malignancies and the diagnosis of mediastinal sarcoidosis. The aim of this study was to review our experience with the cytologic diagnosis of granulomas in patients with pulmonary and extrathoracic malignancies.

Design: We performed a retrospective review of all cytologic diagnoses of granulomas in mediastinal lymph nodes obtained from 9/1/2004 to 3/30/2010 in patients with a concurrent or preceding diagnosis of malignancy. Demographic information, previous malignancy data, symptoms, imaging findings, and follow-up information was extracted through electronic chart review.

Results: 65 patients had cytologically diagnosed mediastinal granulomas; of these 20 (8M, 12F, aged 36-83, mean 60y) had a previously or concurrently diagnosed malignancy: 3 non-small cell carcinoma of the lung (1 adenocarcinoma (ADCA), 1 squamous cell carcinoma (SqCC), 1 NSCC NOS), 3 non-Hodgkin lymphomas, 2 breast carcinomas, 2 gastric ADCA, 2 head and neck SqCC, 1 cervical and 1 anorectal SqCC, 1 hepatocellular carcinoma, 1 thyroid papillary carcinoma, 1 melanoma and 1 malignant fibrous histiocytomas of soft tissues. Malignancy was diagnosed <1 year prior to the diagnosis of mediastinal granulomas in most cases.
The abnormal mediastinal lymph nodes that were sampled by EUS (8 cases) or EBUS (12 cases) were located in descending order in stations 7, 4R, 4L and 11R and 11L and measured 0.8-3 cm (mean 1.6) on CT and had an SUV of 5.4-9.7 (mean 7) on PET. Most patients were asymptomatic and none had a prior history of sarcoidosis. Metastatic disease was clinically suspected in most cases. The granulomas were nonnecrotizing in 15 (all with negative AFB and GMS stains) and necrotizing in 5 cases, two of which showed Histoplasma on GMS stains. Flow cytometry immunophenotyping was performed and was negative in all cases with a history of lymphoma. Follow-up histologic sampling by mediastinoscopy (9 cases) or repeat FNA (2 cases) confirmed the original diagnosis; no malignancies were found. None of the patients had an increase in size of mediastinal lymph nodes or developed mediastinal metastases during a mean two year follow-up period.
Conclusions: EBUS FNA accurately diagnosed the granulomatous disease in all patients with follow-up histologic diagnoses. The significance of sarcoid-like granulomas in patients with malignancies is incompletely understood and deserves further study.

382 Micropapillary Urothelial Carcinoma: Cytologic Features in Exfoliative Urinary Specimens.

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Background: Micropapillary urothelial carcinoma (MUC) is a rare, high grade variant of urothelial carcinoma (UC) that is often invasive into muscle and lymphovascular spaces at presentation and carries a poor prognosis. Extent of micropapillary histology correlates inversely with prognosis. Cystectomy for low stage tumors (pTa, pT1) with micropapillary histology may be appropriate. Recognizing this high grade variant in urinary cytology, the most widely used non-invasive test to detect UC, is important in distinguishing this aggressive tumor from the relatively less aggressive UCs.
Design: A retrospective search for histologically confirmed MUCs with concurrent or near-concurrent urinary cytology was performed. Cases with sarcomatoid differentiation, small cell carcinoma, or mucinous adenocarcinoma were excluded from analysis. All cytologic specimens were prepared using ThinPrep. Cytologic findings, including cell arrangement, nuclear and cytoplasmic features, and N:C were examined.
Results: Twenty-three cases from 22 patients were identified over a 4 year period. Micropapillary clusters and single cells were present in 12 and 23 specimens, respectively. Chromatin was hyperchromatic (n=6), intermediate (n=15), and bland (n=2). Irregular nuclear contours were present in all specimens and nucleoli in 17 specimens. Cytoplasmic vacuoles were evident in 13 cases, including solitary vacuoles (n=6) and multiple vacuoles (n=8); cells in 2 specimens variably demonstrated solitary and multiple vacuolization. All cases demonstrated increased N:C. Corresponding surgical pathology specimens showed varying degrees of micropapillary architecture.
Conclusions: Urinary specimens from patients with histologically confirmed MUC show malignant cells that usually exhibit high grade cytologic features, including marked hyperchromasia, irregular nuclear contours, and clusters with micropapillary architecture. Cytoplasmic vacuoles are also frequently present, a feature not typically seen in UC. These cytologic findings may be useful in managing patients with MUC. Further investigation of urine cytology for diagnostic features distinguishing MUC from other variants is warranted.

383 Detection of In Situ and Invasive Endocervical Adenocarcinoma on Thinprep Pap Test: A Study of 71 Histologically-Proven Cases.

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Background: Incidence of *in situ* and invasive endocervical adenocarcinoma (AIS and ECA) has shown a 25% increase. Data supporting the sensitivity of ThinPrep Pap Test (TPPT) for detection of these lesions is scarce. Herein, we determine sensitivity of TPPT for histologically-proven AIS and ECA with/without associated HSIL and examine discrepancies.
Design: All TPPT (one Pap-stained TP) prior to histologic diagnoses of AIS and ECA over a 6-year-period (2005-to date) were reviewed. TPPT immediately preceding the abnormal histology was defined as the Index Case. Discrepancies in unsatisfactory (Unsat) or negative (Neg) TPPT were evaluated.
Results: 123 TPPT performed in 71 women (age range: 17-70 years; mean: 37) with histologic diagnoses of AIS and ECA were reviewed. Table 1 shows cytology and histopathology correlations for the 71 Index Cases. 65/71 (91.6%) Index TPPT were abnormal. Overall, 41/123 TPPT from 26 women were Unsat (1) or Neg (40). Upon consensus review (RSH, ME) 24 cases (19.5%) from 14 women were true-negative (TN), 2 were Index Cases and 17 cases (13.8%) from 12 women were false-negative (FN), 4 were Index Cases. FN cases preceded histologic AIS and ECA diagnoses by 1 to 34 months. Review diagnoses on FN cases were: AGC/AEC, 13; ASC-H, 2; ASCUS, 2. Reasons for discrepancies included sampling, scant cellularity, lack of traditional morphology of AIS (feathering, palisaded strips, etc.) and ECA (single neoplastic cells, diathesis, etc.) on TP, presence of unusual lesional cytomorphology (small nuclei approximately 7u in diameter with dense chromatin, etc.), and misinterpretation of lesional cells as benign, reactive or metaplastic endocervical or endometrial cells.

AIS and ECA: Correlation of Cytology and Histopathology Diagnoses

	Unsat/Neg	ASCUS/LSIL	HSIL	AGC-EC	AIS	ECA	Total on Cytology
ECA	04	01	01	06	01	06	19
ECA+HSIL	00	00	03	01	00	02	06
AIS+HSIL	01	04	13	07	03	00	28
AIS+LSIL	00	03	00	00	00	00	03
AIS	01	01	04	06	01	02	15
Total on Histology	06	09	21	20	05	10	71

Conclusions: 91.6% of histologically-proven AIS and ECA were diagnosed as abnormal on Index TPPT. Discrepancies were attributable to sampling, lack of traditional morphology of AIS and ECA on TP, and misinterpretation of lesional cells as benign, reactive or metaplastic endocervical or endometrial cells.

384 Immunocytochemistry for MUC4, MUC16, NGAL and ECD as an Adjunct in the Diagnosis of Pancreatic Fine Needle Aspiration Cytology.

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Background: Endoscopic ultrasound guided fine needle aspiration (FNA) of pancreatic mass lesions is a safe method for diagnosis; however, diagnoses rendered as atypical/suspicious for malignancy range from 2% to 29% in various studies. Immunocytochemistry with biomarkers may help in improving sensitivity and specificity within this category. We have identified the expression of four genes, MUC4, MUC16, NGAL (neutrophil gelatinase associated lipocalin) and ECD (ecdysoneless), that are highly upregulated in pancreatic carcinoma as compared to normal pancreatic ducts. In this study, we analyzed their expression in FNA samples in a blinded study at a single center.
Design: Sections from formalin fixed and paraffin embedded cell blocks of FNAs of the pancreas performed at a major tertiary hospital over three consecutive years were reviewed. Unstained sections were immunostained for MUC4 (clone 8G7), MUC16 (clone M11, Dako), ECD (monoclonal), and NGAL (polyclonal) using standard immunohistochemical methods. Anti-MUC4, anti-ECD and anti-NGAL were prepared in our laboratory. Immunostaining was assessed using the H-score (summation of the product of staining intensity and proportion of cells staining). For analysis, any case with an H-score of >0.5 was considered positive. Statistical analysis was done using Medcalc, version 8.1.0.0 for Windows.
Results: The cases were classified using cytomorphologic criteria as carcinoma (57%), benign (9%), atypical/suspicious (20%) and inadequate for immunocytochemistry (14%). On follow-up, all cases diagnosed as carcinoma on cytology were confirmed on biopsy/resection samples. Of the 20% diagnosed as atypical/suspicious, 86% were positive on biopsy/resection and 14% were benign. Overall sensitivity and specificity for the various markers were as follows: MUC4 (72% and 100%), MUC16 (73% and 100%), ECD (85% and 50%) and NGAL (54% and 57%). In cases that were atypical/suspicious on cytology (20%), expression of MUC4, MUC16 and ECD was 100% specific for carcinoma with a sensitivity of 50%.
Conclusions: Immunocytochemistry for MUC4, MUC16 and ECD appears to be a useful adjunct in the classification of pancreatic FNA samples, especially in cases that are equivocal (atypical/suspicious) on cytomorphologic assessment.

385 Incidence and Clinical Outcome of Pregnant Women Diagnosed with ASC-H or HSIL on Antepartum Pap Test.

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Background: According to the 2006 consensus guidelines, management of pregnant women with atypical squamous cells cannot exclude high grade dysplasia (ASC-H) or high grade squamous intraepithelial lesion (HSIL) is determined by the initial colposcopy result. The focus of our study is to examine the incidence and clinical outcome of pregnant patients diagnosed with ASC-H or HSIL on antepartum Pap smear in a high-risk patient population.
Design: We conducted a retrospective review of cervical cytology results in pregnant patients from a large inner-city hospital with a high-risk population from July 2006 to December 2009. Patients were included in the study based on concurrent placenta confirming an underlying pregnancy. Postpartum cytology (cervical smear) and/or surgical pathology results (biopsy or excision) were reviewed.
Results: A total of 2791 patients with antepartum cervical cytology results were identified. Squamous cell abnormality was present in 707 (25.4%), including 341 (12.2%) of atypical squamous cells of undetermined significance (ASCUS), 58 (2.1%) of ASC-H, 273 (9.8%) LGSIL and 34 (1.2%) HGSIL. An analysis on antepartum HSIL or ASC-H results was performed.
Post partum specimens were available in 24 patients with HSIL, 14 (58.3%)of which were classified as high grade by cytology or histology and 2 as ASC-H (Tables 1-2). Follow-up in women with ASC-H (35) included high grade results in 7 (20%) cases and ASC-H in 1 patient. Eight patients (33.3%) with antepartum HSIL and 27 (77%) with ASC-H had LSIL or less on post partum follow-up.

Table 1. Postpartum follow-up results with Histology.

	NEG	CIN 1	CIN 2	CIN 3
HSIL	2(18.2%)	0	7(63.6%)	2(18.2%)
ASC-H	4(30.8%)	6(46.2%)	2(15.4%)	1(7.7%)

Table 2. Postpartum follow-up results with Cytology only

	NILM	LSIL	HSIL	ASC-US	ASC-H
HSIL	1(7.7%)	4(30.8%)	5(38.5%)	1(7.7%)	2(15.4%)
ASC-H	12(54.5%)	3(13.6%)	4(18.2%)	2(9.1%)	1(4.5%)

Conclusions: In a high risk patient population, the number of pregnant women diagnosed with squamous cell abnormality is higher. There is no difference in incidence of a high grade lesion on a postpartum sample in the study group when compared to

non-pregnant women. Although ASC-H is a poorly reproducible diagnosis, it has a significant predictive value for an underlying high grade lesion. Our data shows that significant number of patients diagnosed with ASC-H or HSIL on antepartum Pap smear have a persistent high grade lesion postpartum. Possible explanation for follow-up results less than HSIL include regression, sampling or interobserver variability.

386 Digital Images in Gynecologic Cytology Reports: An Incentive To Downgrade.

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Background: The use of digital images in cytology and surgical pathology reports is a growing trend but by current guidelines is considered entirely optional. There has been little investigation of the effects, including potential unforseen consequences, of requiring the inclusion of such ancillary material in anatomic pathology reports, and, more specifically, gynecologic cytology reports.

Design: A recent management change in a large consulting supplier of ThinPrep liquid-based gynecologic cytology specimens for our institution resulted in a requirement for digital photographs in gynecologic cytology reports for the following interpretive categories: ASC-H, LSIL, LSIL-H, and HSIL. We reviewed 1425 liquid-based gynecologic cytology cases submitted for pathologist review at our institution, all from the same consulting group, occurring over a consecutive time period: 713 of these fell prior to the new requirement and 712 followed the start of the photograph requirement. We evaluated these cases based on the number of pathologist upgrades and downgrades and whether these changes resulted in a change in the picture status.

Results: Of the specimens reviewed by a pathologist following the photograph requirement, 32 cases were upgraded from NILM or ASC-US to a photograph-requiring interpretation of ASC-H, LSIL, LSIL-H, or HSIL (compared with 40 before the requirement), and 37 were downgraded from a photograph-requiring interpretation to NILM or ASC-US (compared with 20 before the requirement). The remaining specimen interpretations were either changed without changing the need for a photograph (129 cases after the requirement versus 96 cases before) or were left unchanged by the pathologist (514 cases after the requirement versus 557 cases before). A chi-square analysis of these results yielded a p-value of 0.0058. The ASC:SIL ratio increased from 1.07 to 1.45 after the start of the photography requirement.

Conclusions: Inserting a picture into the cytology report requires additional effort on the part of the pathologist. Therefore, there is an incentive to not add a picture either by downgrading or by not upgrading an interpretation. Our results show a corresponding shift in gynecologic cytology interpretations in our institution.

387 Changing Trends of Fine Needle Aspirate Diagnosis of Lung Neoplasm in the Face of Customized Patient Management Approach. Are We Going to Step Up?

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Background: Transthoracic image guided fine-needle aspiration (FNA) is one of the mainstay and utterly useful initial diagnostic modalities for acquiescent focal lung lesions. A large body of literature is available regarding accessibility, yield, sensitivity, specificity and accuracy of lung FNA but recent expectations for profiling classified tumours are becoming an important and desirable diagnosis to tailor tumour specific targeted therapies. The objectives of our study include overall evaluation and efficacy of FNA in the diagnosis of neoplastic lung lesions and to observe the evolvement of diagnostic reporting trends from traditional to more specific classification of tumour and molecular testing phased in at our institution.

Design: Cytology reports of FNA performed on 2206 patients with lung lesions over a 3 year period (2007-2009) were retrieved from the archives of cytopathology of the Ottawa Hospital. During the study period, 517 cases with histologically proven non-small cell carcinoma (NSCLC) diagnoses were identified and evaluated for cytological-histological correlation. Sections of cell blocks of FNA samples of adenocarcinoma cases were tested for EGFR exon 19 and exon 21mutations.

Results: Patients' age ranged from 31 to 90 years with a male: female ratio of 1.69:1 and collectively a diagnosis of neoplasm was rendered for 75.2% for 2206 FNA procedures performed whereas 2.5% were suspicious for malignancy, 4.8% atypical, 14% negative for malignancy and 3% were non-diagnostic. The sensitivity was 100% as all histologically proven non-small cell carcinomas had positive cytology. A specific diagnosis for adenocarcinoma (AdCa) and squamous cell carcinoma (SqCCa) improved from 33% in 2008 to 42% in 2009 in proven NSCLC cases and a diagnosis of atypical cells decreased from 11.4% to 6.7% in all malignant cases. Accuracy rate for SqCCa was 100% and for adenocarcinoma was 98%. 10.3% of adenocarcinoma FNA samples tested for EGFR mutations were positive.

Conclusions: Relatively less invasive, time efficient and cost effective FNA samples obtained by experienced interventionists are optimal to deliver classified tumor diagnosis in a significant number of non-small cell carcinoma cases. In addition, these samples if preserved properly, can be utilized for immunohistochemical studies to further refine the diagnosis and to perform molecular diagnostic techniques to deliver customized oncological chemotherapeutic patient management.

388 Consensual Review Minimizes the Diagnosis of "Follicular Lesion of Undetermined Significance" and Improves Reproducibility and Cyto-Histologic Concordance.

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Background: There has been considerable variation among both pathologists and institutions in using the terminology of "atypia of undetermined significance/follicular lesion of undetermined significance" (AUS/FLUS). The Bethesda System for Reporting Thyroid cytology (BSRTC) defines the diagnostic criteria for AUS/FLUS and advises that AUS/FLUS should not be used indiscriminately. The current study was conducted to investigate if consensual review may minimize AUS/FLUS and may also improve reproducibility and cyto-histologic concordance.

Design: A SNOMED search of the electronic pathology database for the period of January 1998-June 2010 retrieved a total of 217 aspirates in which the original interpretation of AUS/FLUS was rendered and followed by hemi- or total thyroidectomies. The retrieved aspirates were retrospectively reviewed using the BSRTC as a guideline. A group of reviewers who were blinded to the corresponding histologic findings simutaneously evaluated slides of each aspirate at a multi-headed microscope and a consensus diagnosis was reached at the end of the group review. Interobserver agreement was calculated by dividing diagnosis of the majority by the total. The Cyto-histologic correlation was then performed between the consensus diagnoses and the corresponding histologic diagnoses. This is an ongoing study and a total of 50 aspirates have been reviewed.

Results:

CORRELATION BETWEEN CONSENSUAL CYTOLOGIC INTERPRETATION AND HISTOLOGIC DIAGNOSIS

		Histologic Diagnosis				
Cytologis Diagnosis	Interobserver Agreement (%)	benign	follicular adenoma	follicular carcinoma	PTC	TOTAL(%)
non-diagnostic	100	2	-	-	-	2 (4)
benign	90.5	24	2	-	-	26 (52)
AUS/FLUS	71.6	5	3	2	1	11 (22)
FN/SFN	81.8	2	5	1	2	10 (20)
PTC	100	-	-	-	1	1 (2)
TOTAL	_	33	10	3	4	50 (100)

FN/SFN: positive/suspicious for follicular neoplasm; PTC: papillary thyroid carcinoma

The data generated from 50 cases demonstrates that using BSRTC as a guideline, consensual review of aspirates previously categorized as AUS/FLUS: 1) markedly reduces the diagnosis of AUS/FLUS (11 out of 50) without compromising diagnostic accuracy. 2) provides a better cyto-histologic concordance and archieves a diagnostic accuracy of 89.2%. 3) The average level of interobserver agreement of 88.8% is archieved

Conclusions: Using BSRTC as a guideline, consensual review of aspirates previously categorized as AUS/FLUS plays a substantial role in reducing unnecessary surgical intervention by 48%.

389 Root Cause Analysis for False Suspicion of Papillary Thyroid Carcinoma: Review of 22 Cases.

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Background: The Bethesda Sysyem for Reporting Thyroid Cytopathology (BSRTC) demonstrtes the criteria of suspicious for malignancy (SFM) and recommends judicious use of this diagnostic category so that an ideal positive predictive value can be achieved. Suspicious for papillary thyroid carcinoma (SPTC) represents the majority of SFM cases. The current study was undertaken to address the root causes for false SPTC.

Design: We retirieved a total of 22 aspirates in which the original cytologic interpretation of SPTC was rendered and the corresponding histology revealed non-PTC. The retrieved aspirates were retrospectively reviewed along with the corresponding surgical specimens. Morphological features were assessed and recorded.

Results: The corresponding histology of the 22 aspirates interepreted as SPTC revealed 16 nodular hyperplasia and 6 follicular adenoma. Ten aspirates were moderately cellular and 12 aspirates were hypocellular. Ten aspirates contained moderate amount of colloid. Scant or no colloid presented in 12 aspirates. Various proportions of monolayer honeycomb sheets, intact follicles and microfollicles were appreciated among all aspirates. Focal syncytium was seen in one aspirate. Conspicuous fragments of fibrocollagenous tissue with entrapped follicular cells were detected in one aspirate. Elongate or spindel cells presented in 8 aspirates and some were accompanied by histiocytes. Thin, intranuclear grooves presented focally in all aspirates and some grooves appeared incomplete. The grooves were noted mainly in rounded nuclei, as well as nuclei of the entrapped follicular cells and the elongate or spindle cells aforementioned. Rare intranuclear inclusions were detected in two aspirates. Nuclear paller was rarely seen. Presence of mild nuclear atypia including nuclear molding, nuclear enlargement, irregular nuclear membrane was minimal among all aspirates.

Conclusions: Based on retrospective cytologic review and histology correlation, the following contributed to false suspicion of SPTC:

1) Fragments of fibrocollagenous tissue with entraped follicular cells were micinterpreted as fibrovascular cores.

2) Misinterpretation of the the enlongate or spindel cells that actually represented atypical cyst lining cells.

3) Overinterpretation of suboptimal intranuclear grooves noted in the nuclei with minimal diagnostic features of PTC

4) Overinterpretation of rare intranuclear inclusions in the absence of other diagnostic features of PTC

390 Utility of Molecular Testing in Fine Needle Aspiration and Needle Core Biopsy Diagnosis of Renal Mass Lesions.

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Background: Fine-needle aspiration (FNA) and needle core biopsy (NCB) can provide essential information for the diagnosis and management of renal masses. However, reported disadvantages of these diagnostic modalities include high unsatisfactory rates, false negative diagnoses and difficulty in subclassifying renal tumors. The later

may be due to inadequate sampling and/or lack of helpful ancillary studies. Our aim was to review the value of diagnostic molecular testing on FNA and NCB material from renal tumors.

Design: A total of 120 cases of renal FNA were obtained from the cytopathology archives at our institution from 2000 to 2010. Complementary NCB was performed on 20 of these renal masses. Diagnoses were categorized as malignant, atypical, benign or unsatisfactory. Cytological diagnoses were correlated with subsequent surgical pathology findings in 42 available cases. Molecular tests performed included fluorescence in-situ hybridization (FISH) with UroVysion probes (for chromosomes 3, 7, 17 & 9p21 band), trisomy and monosomy for chromosomes 1,7 and 17 and loss of heterozygosity (LOH) for deletion (del) 3p in total of 12 cases with sufficient cell block or NCB material from 2008-2010.

Results: Solid lesions (n=101) were classified as unsatisfactory in 4 cases (4%), benign in 11 (11%), atypical in 14 (14%), and malignant in 72 (72%) cases. For cystic lesions (n=19), 16 (84%) were benign and 3 (16%) atypical. Malignant cases included 25 renal cell carcinoma (RCC) with subtyping, 27 RCC not otherwise specified (NOS), 4 oncocytic neoplasms, 6 urothelial carcinomas (UC), 1 neuroendocrine carcinoma, 3 metastatic carcinomas, and 6 B-cell non-Hodgkin lymphoma. Molecular testing was positive in 7 of 12 cases (58%). FISH with UroVysion was positive (trisomy 3, 7 and 17) in 2 cases, including1 case of UC and 1 case of papillary RCC. FISH was also positive for monosomy 1, 7, 17 in 1 clear cell RCC. UroVysion was negative in 1 case of poorly differentiated UC. LOH studies for del 3p were performed in 8 cases and was positive in 4 (50%) cases of RCC clear cell type, negative in 2 (25%) cases of RCC (NOS), and negative in 2 atypical cases (25%).

Conclusions: This data suggests that molecular testing performed on FNA and NCB may enhance the diagnosis of specific renal neoplasms. The greatest drawback is limited sampling which results in inadequate material for molecular testing. On-site cytological evaluation and complementary NCB may assure adequate sampling.

391 Cytological Evaluation of "Bloody" Nipple Discharge with Histopathological Correlation: Study of 50 Cases.

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Background: Nipple discharge can be serous, milky, mucoid, turbid or bloody. **Bloody** nipple discharge (BND) is particularly alarming, and is considered most likely to be associated with a pathological (rather than physiological) cause. Data on BND cytology (BNDC) with histopathological correlation is scarce.

Design: Excisional biopsies (including micrdochectomies), 2000-to date, submitted with BND as clinical information, and in which there had been prior BNDC were identified and reviewed. Cytological evaluations were classified as Positive (Pos), Papillary (Pap), Atypical (Aty), Negative (Neg) or Unsatisfactory-Acellular (Unsat).

Results: 50 cases with 55 BNDC (5 cases with 2 specimens <8 months apart) were studied. Mean age of patients was 50 (range: 14-87). BND was unilateral in all 50 patients (right: 28, left: 22). Histopathology diagnoses were invasive carcinoma (Inv Ca): 5; ductal ca *in situ* (DCIS): 5; atypical hyperplasia (Aty): 2; papilloma: 16 (32%, single most common cause of BND); other benign (Oth): 22. In 4 BNDC cases, the 1st specimen was Unsat or Neg. In 1 case, both BNDC were Pap. Final cytology diagnoses were Pos: 3; Pap, 13; Aty: 4; Neg: 25; and Unsat: 5.

Bloody Nipple Discharge: Correlation of Final Cytology and Histopathology Diagnoses

	Invasive Ca	DCIS	Atypical	Papilloma	Other	Total, on Cytology
Positive	1	0	1	1	0	3
Papillary	2	2	0	4	5	13
Atypical	0	0	0	2	2	4
Negative	2	3	1	7	12	25
Unsatisfactory	0	0	0	2	3	5
Total, on Histopathology	5	5	2	16	22	50

Table shows correlation of cytology and histopathology diagnoses. Cytology preps included direct smears in 51, ThinPrep in 2, and both in 2. Direct smears were stained *via* Pap stain (89%) or DiffQuik (11%). Significant air-drying artifact was seen in 15% (8/55) specimens. Cytology preps were Unsat in 13% (7/55) specimens. Cytology features evaluated included background, necrosis, rbc's, macrophages, cellularity, cell clusters, nuclear atypia, columnar cells and apocrine cells. 25 Neg BNDC predominantly showed rbc's and macrophages. Necrosis was present in 1 case of sclerotic papilloma. Apocrine cells were identified in 1 case of DCIS.

Conclusions: In this series, sensitivity of BNDC was 20%, specificity was 96%, accuracy was 88%, and precision was 33%. Factors most likely leading to false-negative and false-positive results in cytology were unsatisfactory smears and air-drying, respectively. Presence of necrosis and apocrine cells may be misleading. BND was more commonly associated with benign lesions in 76% (38/50) of cases in this series.

392 Comparison of HPV Detection Technologies; Hybrid Capture 2 (Qiagen), Full-Spectrum HPV (Genoid), Genoid Molecular Beacon Real-Time HPV Assay with Genotyping by Linear Array (Roche) and Genoid HPV ELISA Genotyping Assay in an Irish Colposcopy Population.

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Background: Cervical screening programmes are moving towards HPV testing as part of the screening process and as triage for colposcopy. We evaluated 3 HPV detection and 2 genotyping methods on liquid based cytology specimens from a colposcopy population. This study forms part of the AutoCast Consortium funded under the EU 7th framework and is supported by the Irish Cervical Screening Research Consortium CERVIVA formed under the Health Research Board.

Design: Cytology specimens from 241 women with greater than 2 persistently abnormal smears were recruited through the Coombe Women and Infants University Hospital, Dublin. Smears were taken at first visit and prior to any procedure. Patients were examined colposcopically and a biopsy or Lletz performed. Cytological diagnoses were made using BSCC guidelines. HPV DNA was detected by Hybrid Capture (hc2) for 13 high-risk HPV types, Full-Spectrum HPV (FS-HPV) for 49 high and low-risk types and Molecular Beacon Real-Time HPV assay (MB-RTHPV) for 16 high and low-risk types. HPV genotyping was performed using Linear Array HPV Assay (LA) and HPV ELISA assay. Histology results were available for 186 cases.

Results: HPV was detected in 83.3% (195/234), 82.6% (199/241), and 70.1% (169/241) of cytology specimens by hc2, FS-HPV and MB-RTHPV HPV DNA detection assays. 198 specimens were valid by all three HPV detection assays. The sensitivity of the assays detection of HPV in cases with CIN2+ cytology were 100%, 99% and 95% for hc2, FS-HPV and MB-RTHPV assays with positive predictive values (PPV) of 94%, 93% and 94%. The sensitivity of the assays for the detection of HPV in cytology specimens that had a CIN2+ result by histology were, 98%, 99% and 94% for hc2, FS-HPV and MB-RTHPV assays with PPV of 94%, 93% and 97%. The most common HPV genotypes were 16, 31, 33, 58, 42, 61 and 53 for the LA assay versus 16, 31, 33, 42, 58, 51 and 66 by HPV ELISA.

Conclusions: The FS-HPV and MB-RTHPV show comparable sensitivity and have a similarly high PPV as the hc2 assay for HPV detection in CIN2+ patients. HPV genotype distribution was similar for all types with the exception of HPV18 which was detected more frequently using LA.

393 Utility of PAX8 and PAX2 Immunohistochemistry in the Diagnosis of Renal Cell Carcinoma in Cytology Specimens.

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Background: The diagnosis of metastatic renal cell carcinoma (RCC) in cytology specimens may be difficult to confirm on the basis of cytomorphology alone. Often, immunohistochemistry serves as an important adjunct in confirming this diagnosis. Recently, PAX2 was shown to be useful in this regard. As there are no reports to date that examine the role of PAX8 in the cytologic diagnosis of RCC, we sought to investigate and compare the diagnostic utility of PAX8 and PAX2.

Design: First, the performance of PAX8 immunohistochemistry was verified using a tissue microarray (TMA) composed of 30 clear cell RCCs, 17 papillary RCCs, and 7 chromophobe RCCs. Next, the pathology database was searched from 2000 to 2010 for cases in which a cytologic diagnosis of RCC was rendered in patients with histologically confirmed disease based on primary resections and/or biopsies of metastases. Twenty-four cases (17 fine needle aspirates and 4 effusions) were identified. The associated histologic diagnoses were clear cell RCC (14 cases), papillary RCC (4 cases), papillary RCC with sarcomatoid transformation (1 case), pure sarcomatoid RCC (1 case), and RCC of unknown subtype (4 cases). Immunohistochemistry using rabbit polyclonal antibodies directed against PAX8 (1:200 dilution; ProteinTech, Chicago, IL) and PAX2 (1:100 dilution; Invitrogen, Camarillo, CA) was performed on cell block sections prepared from these cases.

Results: PAX8 immunoreactivity was seen in at least 10% of the tumor cells in all cases of RCC in the TMA. Next, PAX8 positivity was seen in 21 (88%) of 24 cytology cases. In 17 of the 21 PAX8(+) cases, greater than 50% of the tumor cells exhibited nuclear immunoreactivity for PAX8. In the other four PAX8(+) cases, at least 10% of the tumor cells were immunopositive. For comparison, the 24 cytology cases were also immunostained for PAX2. PAX2 positivity was seen in 20 (83%) of 24 cases. In 14 of these 20 PAX2(+) cases, over 50% of the tumor cells were PAX2(+). In the other six PAX2(+) cases, at least 10% of the tumor cells were immunoreactive for PAX2. Overall, immunoreactivity for both PAX8 and PAX2 was seen in 19 (79%) of 24 cases. Finally, positivity for either of the two markers was detected in 22 (92%) of the 24 cases.

Conclusions: Our results demonstrate that both PAX8 and PAX2 are useful markers for identifying metastatic RCC in cytology specimens. While PAX8 shows a slight increase in sensitivity over PAX2 in RCCs, the highest sensitivity and therefore greatest utility lies in using both markers for confirming a diagnosis of RCC.

394 Detection of Her-2 Neu Gene Expression in Cytological Specimen.

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Background: Documenting her 2 neu gene copies by fluorescence in situ hybridization has become standard practice for all invasive breast cancers. Identifying the presence of increased her 2 neu gene copy number can greatly impact the type of treatment and ultimate prognosis in breast cancer patients. Such studies are usually conducted on the original core biopsy or excisional biopsy of the primary breast cancer; however, on occasion patients present with metastatic tumors in which case the diagnosis is usually rendered on a cytological specimen. The current study was designed to assess whether these samples can yield adequate and accurate results.

Design: The UAMS Molecular Pathology Laboratory database was searched to identify all cytology specimens submitted for fluoresce in situ hybridization (FISH) for her 2 neu. The cases were stratified according to their source, primary site or metastatic lesions to solid organs, i.e. liver, bone, or effusions. The result were analyzed and compared to paired histological sections of the core biopsies and excisions whenever possible.

Results: From January 2003 to March 2010, our laboratory performed FISH for her 2 neu on 64 cell blocks from cytological specimens including, 10 fine needle biopsies (FNA) obtained from the primary tumor site and 54 FNAs from metastatic sites, including pleural and peritoneal effusions. Five cases were rejected due to low cell number while 1 case failed to return a result due to a technical issue. The test was successfully completed in the remaining 59 cases. A total of 35 cases were from FNA

of various metastatic sites including lymph nodes (5) liver (8), lung (4) soft tissue (5), neck masses (3), and brain mass (1), and bone aspirates (9). Ten cases were reported as amplified, two cases were borderline and the remaining cases were negative. Our laboratory also performed FISH on 19 effusions, including 17 pleural effusions, one ascites, and one pericardial effusion. Over the same time period, our laboratory also completed FISH studies on 1313 histological sections of core biopsies or excisions. Of these, 200 (15.8%) cases were reported as positive, 19 cases (1.5%) borderline and 1043 cases negative while 52 cases (4%) were reported as inadequate due to insufficient number of cancer cells, or due to technical difficulty, most likely secondary to problems related to fixation.

Conclusions: Fluorescense in situ hybridization can be performed successfully on cytological specimens. The rate of insufficiency is slightly higher 7.7 as comparison to 4% seen from histological sections.

395 DOG1: Utility in Diagnosing Gastrointestinal Stromal Tumors on Fine Needle Aspiration.

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Background: Gastrointestinal stromal tumors (GIST) are the most common mesenchymal tumors of the GI tract, and the majority contain *KIT* or *PDGFRA* activating mutations. Fine needle aspiration biopsy (FNAB) is a valuable tool in the diagnosis of GIST, and may allow for pre-operative therapy with tyrosine kinase inhibitors (TKI). Because of the morphologic diversity of these tumors, routine diagnosis of GIST often relies on C-Kit immunohistochemical staining in addition to morphologic findings. However, up to 15% of GISTs are C-Kit negative. Antibodies with increased sensitivity and specificity for detection of C-Kit negative GIST cases could be of value, especially since some of these cases may also benefit from TKI therapy.

Design: Immunohistochemical staining for DOG-1, C-kit (CD117), and protein kinase C theta (PKCθ) was performed on FNA cell block preparations representing 18 GISTs, 17 leiomyosarcomas, 16 melanomas, 16 schwannomas and 11 adenoid cystic carcinomas (ACC).

Results:

	GIST	Leiomyosarcoma	Melanoma	Schwannoma	ACC
DOG-1	18/18 (100%)	0/17 (0%)	0/16 (0%)	0/16 (0%)	0/11 (0%)
C-Kit	13/18 (72.2%)	1/17 (5.9%)	4/16 (25%)	0/16 (0%)	10/11 (90.9%)
PKCθ	6/18 (33.3%)	1/17 (5.9%)	5/16 (31.6%)	0/15 (0%)	1/10 (10%)

DOG-1 was found to have 100% sensitivity and 100% specificity in diagnosis of GIST. C-Kit demonstrated 72.2% sensitivity and 75.0% specificity, and PKCθ showed 33.3% sensitivity and 88.3% specificity. When only spindle cell neoplasms were considered (adenoid cystic carcinomas excluded), the specificity of C-Kit increased to 91.7%. Of interest, all C-Kit negative cases showed DOG-1 positivity.

Conclusions: DOG-1 is the most sensitive and specific of the three markers for the diagnosis of GIST in cell blocks, and may be of particular use in the diagnosis of C-Kit negative GIST. In addition, DOG-1 helps in differentiating GIST from neoplasms such as ACC and melanomas which have a high incidence of C-Kit positivity, and may be challenging in cases of metastatic disease.

396 Banking of Fine Needle Aspiration Biopsies for Future RNA Based Molecular Testing.

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Background: Fine needle aspiration (FNA) biopsy is minimally invasive, cost-effective and also allows for sampling of diseased cells (i.e. metastatic and pre-malignant) that may not be accessible through excisional biopsy. Ancillary molecular testing is becoming routine in Surgical Pathology, but there have been limited reports describing the utility of FNA biopsies for this purpose. Quality control (QC) and test validation requirements for development of molecular tests impose a need for access to preexisting clinical samples. Banking FNA biopsy specimens would allow for innovative genomic research and development of novel molecular assays from cytology specimens. Here, we evaluated cryopreservation of FNA specimens as a method of maintaining cellular morphology and RNA integrity in banked tissues.

Design: FNA specimens were collected from tumor resections received in Surgical Pathology. Syringe contents were immediately deposited into either cryopreservation media (n=25) or as a control, the RNA stabilizing reagent RNAlater (n=13(Applied Biosystems, Foster City, CA)). RNAlater specimens were stored at 4°C for less than one month per manufacturer's instructions. Specimens for cryopreservation were processed using controlled rate freezing and stored for up to 27 weeks in the vapor phase of liquid nitrogen. At varying time intervals cryopreserved specimens were quickly thawed at 37°C then washed in fresh media. A portion of each sample was used for cytospin preparation for morphological evaluation. RNA was extracted from the remaining portion and was assessed for integrity using the Agilent Bioanalyzer and RNA integrity number (RIN) software tool (Agilent Technologies, Inc., Santa Clara, CA).

Results: Cryopreserved specimens showed good cell morphology, and in many cases yielded intact RNA. Factors affecting RNA integrity included prolonged sample processing delays ($p<0.001$) and sampling of necrotic cells ($p<0.05$). When samples were processed expeditiously there was no difference in RNA quality between cryopreserved and RNAlater specimens ($p=0.6$). Additionally, there was no correlation between the total time samples were frozen (tested up to 27 weeks) and RNA integrity ($r=0.04$).

Conclusions: With careful handling, FNA specimens can be stored in a manner that maintains cellular morphology and RNA integrity necessary for studies in gene expression. Although not tested here, cryopreservation is the only method that might also maintain cell viability. In addition to addressing QC and test validation needs, cytology banks will be an invaluable resource for future molecular research studies.

397 Peritoneal Washing Cytology in BRCA Mutation-Positive Patients Undergoing Risk-Reducing Salpingo-Oophorectomies.

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Background: Peritoneal washing cytology (PWC) obtained at the time of risk-reducing salpingo-oophorectomy (RRSO) in BRCA mutation-positive patients has been recommended for detection of occult primary peritoneal carcinoma. However, only a few small series have been reported, and the prognostic significance of their positive PWCs remains uncertain. To further evaluate the utility of PWC in this group of patients, we reviewed PWCs from 117 patients undergoing RRSO at our institution and correlated the results with surgical pathology findings.

Design: Records of 129 PWCs from 126 BRCA mutation-positive patients undergoing RRSO at MD Anderson Cancer Center between 2000 and 2010 were obtained. Slides were available for review for 120 PWCs from 117 patients (3 patients had 2 PWCs each). Cytopathologists, blinded to the RRSO histopathologic diagnoses, categorized the PWCs as benign, atypical, suspicious for malignancy, or malignant. The presence of endosalpingiosis/Mullerian metaplasia and psammomatous calcifications was also noted. These results were correlated with the RRSO histopathologic diagnoses.

Results: PWCs from 113 patients were benign. Of the remaining 4 patients, 2 had PWCs classified as atypical, 1 as suspicious for malignancy, and 1 as malignant. The corresponding RRSO histopathologic findings of the 2 atypical PWCs were endosalpingiosis and adenofibroma in one case, and showed no abnormalities in the other case. Both patients with suspicious or malignant PWCs had RRSO histopathologic diagnoses of endometriosis and endosalpingiosis and, therefore, were suspected of harboring occult peritoneal carcinoma. Nine patients had abnormal tubal or ovarian histologic findings that included tubal hyperplasia with atypia (4 patients), tubal high-grade serous carcinoma (2 patients – one with tumor limited to mucosa and the other with tumor extending into submuscosa), and ovarian serous tumor of low malignant potential (3 patients). All 9 of these patients, however, had benign PWCs.

Conclusions: PWC has the potential to detect occult peritoneal carcinoma in BRCA mutation-positive patients. The clinical significance of a positive PWC without abnormal RRSO histology remains unclear and will require long term follow-up for determination. The benign cellular constituents in the PWCs from our patients found to have occult carcinomas are not surprising, given the small size and limited extent of their cancers.

398 Napsin-A Expression in Small Cell Carcinoma of the Lung: A Cytologic Study.

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Background: Napsin-A is an aspartic proteinase involved in surfactant protein B maturation. It is overexpressed in pulmonary adenocarcinomas (PA), and is a useful diagnostic alternative to thyroid transcription factor-1 (TTF-1). TTF-1 is also expressed by small cell carcinoma of the lung (SCCA) and facilitates its diagnosis. While Napsin-A expression has been examined in PA and pulmonary squamous cell carcinoma, its expression in SCCA of lung has not been fully evaluated. We examined Napsin-A expression in 36 cytologically confirmed cases of SCCA to determine if its expression paralleled that of TTF-1. To date ours is the largest cytologic series of lung SCCAs examined for Napsin-A expression.

Design: Thirty-six (36) primary and metastatic SCCAs diagnosed on fine needle aspiration (FNAB) or pleural effusion, with corresponding cell blocks, were identified. Patients ranged in age from 43 – 87 years (mean 57 years) with 20 males and 16 females. FNABs of mediastinal masses (n=5), liver masses (n=3), a subcutaneous nodule (n=1), lung masses (n=6), axillary, cervical and mediastinal lymph nodes (n=20) and 1 pleural effusion were immunostained with Napsin-A, TTF-1, pancytokeratin and at least one of three neuroendocrine (NE) markers (synaptophysin, chromogranin, CD56).

Results: The immunohistochemical results are summarized in Table 1.

Napsin-A, TTF-1 and Neuroendocrine Marker Expression in Small Cell Carcinoma of Lung (n=36)

Result	Napsin-A	TTF-1	Synaptophysin	Chromogranin	CD56	Pancytokeratin
Positive	0 (0%)	35 (97%)	29 (81%)	17 (47%)	23 (64%)	35 (97%)
Negative	36 (100%)	1 (3%)	3 (8%)	10 (28%)	1 (3%)	0 (0%)
Not Tested	0 (0%)	0 (0%)	4 (11%)	9 (25%)	12 (33%)	1 (3%)

All 36 cases (100%) of SCCA were Napsin-A negative. Napsin showed focal positivity in macrophages as well as type II pneumocytes. All 36 (100%) cases expressed at least one or more NE marker; 35/36 (97%) were pancytokeratin-positive and 35/36 (97%) TTF-1 positive. The TTF-1-negative SCCA was positive for NE markers and pancytokeratin and showed cytomorphologic features of SCCA.

Conclusions: While Napsin-A is frequently expressed in PA it is almost never expressed by SCCA of the lung. Therefore, Napsin-A should not be used in the cytologic diagnosis of SCCA, other than as an exclusionary parameter. However, in situations where it is cytologically challenging to differentiate SCCA from PA (especially since both are TTF-1-positive), a positive Napsin-A would favor PA, and would therefore prove useful in distinguishing the two. Larger studies are needed to examine Napsin-A expression in other NE lung tumors.

399 Desmoglein-3 Is Inferior to Cytokeratin 5 and p63 in the Cytologic Diagnosis of Squamous Cell Carcinoma (SQC) of the Lung.

SR Lauer, MT Siddiqui, C Cohen, MD Reid. Emory University Hospital, Atlanta, GA.

Background: Current treatment strategies for lung carcinoma are dependent on the accurate histologic and cytologic classification of these tumors. Several immunohistochemical (IHC) stains have emerged as frontrunners in their diagnosis and subtyping. One such IHC stain is desmoglein-3 (DSG-3), a calcium-binding transmembrane glycoprotein component of desmosomes in vertebrate epithelial cells.

It has recently been shown to have a high sensitivity and specificity for the diagnosis (Dx) of lung SQC. We examined DSG-3 in 43 histologically proven lung carcinomas and compared its performance to that of cytokeratin 5 (CK5) and p63, well-known markers of SQC.

Design: A total of 43 resected/biopsied histologically confirmed lung adenocarcinomas (AD) (25), SQC (13) and poorly differentiated carcinomas (PDC) (5) with previous FNA and adequate cell blocks were selected for IHC staining with SQC markers DSG-3, CK5 and p63. Staining was graded as (0), negative; focal, <30% of cells with cytoplasmic staining; and diffuse, ≥ 30% of cells staining. Based on IHC findings a cytologic Dx was rendered and compared to the gold standard histologic Dx.

Results: The results are summarized in Table 1.

Results of IHC Staining with DSG-3, CK5 and P63

Stain	DSG-3	CK5	p63	CK5 & p63
Dx	+	+	+	+
AD (n=25)	1 (4%)	4 (16%)	6 (24%)	0 (0%)
SQC (n=13)	6 (46%)	12 (92%)	10 (77%)	10 (77%)
PDC (n=5)	1 (20%)	1 (20%)	1 (20%)	1 (20%)
Total (n=43)	8 (19%)	7 (16%)	17 (33%)	11 (26%)

All histologically proven SQC's were confirmed as such on IHC. DSG-3 was focally positive in one lung AD and in 46% of lung SQC. When positive in SQC, staining was always focal, weak and difficult to interpret unlike CK5 and p63, which were diffusely and strongly positive. CK5 and p63 were both positive in 77% of lung SQCs. CK5 and p63 were never both positive in AD. Of the 43 tumors, a single PDC was the only tumor that was strongly and diffusely DSG-3 positive. This tumor was also strongly positive for CK5 and p63, confirming a cytologic diagnosis of SQC.

Conclusions: DSG-3 is a less sensitive IHC marker of squamous differentiation than CK5 and p63. The latter both stain SQC strongly and diffusely; DSG-3 is a weak and patchy cytoplasmic stain and is sometimes difficult to interpret. Because of its negativity in the majority of AD it may have limited usefulness in the cytologic distinction between SQC and AD. Compared to CK5 and p63, DSG-3 is a far less definitive marker of squamous differentiation and is not recommended for routine tumor diagnostics, unless sensitivity improves.

400 Comparison of Ultrasound-Guided FNA with CNB in the Evaluation of Thyroid Nodules.

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Background: Studies comparing the diagnostic efficacy of core needle biopsy (CNB) with fine needle aspiration (FNA) of the thyroid are relatively few and have shown conflicting results.

Design: Diagnostic results from 95 cases of concurrent US-guided FNA and CNB of thyroid nodules were retrospectively evaluated. Both procedures were performed in an outpatient setting by one radiologist, utilizing 20 gauge needles for FNA, followed by CNB using Temno 20 gauge core needles with direct US guidance. All cases were interpreted by one pathologist with an interest in thyroid disease using standard diagnostic criteria, accepting that adenomatoid changes may be seen in part within cellular hyperplastic nodules. The cytological results were categorized as nondiagnostic, benign, indeterminate/abnormal, or positive, and the interpretations of the CNB were subsequently similarly categorized. The diagnostic accuracy for both techniques was assessed within the specific categories, utilizing Pearson's Chi-square test.

Results: For FNA, 24 cases were nondiagnostic, 60 were benign, 6 indeterminate/atypical, and 5 cases were positive (all papillary carcinoma). For CNB, 29 cases were nondiagnostic, 50 were benign, 13 indeterminate/atypical, and 3 positive (all papillary carcinoma). Identical diagnoses by both methods were achieved in 48/95 cases to include: 36 benign, 3 positive, 9 nondiagnostic. CNB was diagnostic in 64 cases (67%), and FNA in 65 cases (68%). Sufficient material for diagnosis from at least one technique was provided in 86 cases (91%). In our study, all 5 carcinomas were diagnosed on FNA, with only 3 of these called on CNB. Discordant cases included 4 in which the FNA was benign and the CNB indeterminate, suggesting that adenomatoid hyperplastic nodules lesions are more difficult to diagnose accurately on CNB, possibly due to a more limited sample. Comparing both techniques, FNA was significantly better at establishing a definitive diagnosis than CNB in this study ($P < 0.0001$).

Conclusions: Overall diagnostic sensitivity for CNB and FNA was similar in this study with an improved diagnostic rate with both techniques combined. However, FNA remains a significantly better technique for specific categorization of thyroid nodules. Furthermore, CNB offers no additional diagnostic value in distinguishing cellular (adenomatoid) hyperplastic nodules from true follicular neoplasms. Although this may be due to the more limited nature of the sample provided by CNB, this also emphasizes the need for more clearly defined criteria for diagnoses in thyroid CNB.

401 Significance of Positive Pelvic Washing Cytology in Patients with Endometrial Cancer.

BB Lee, MH Roh, CM Johnston, SM Knoepp. University of Michigan, Ann Arbor.

Background: The 2010 American Joint Committee on Cancer (AJCC) guidelines no longer utilize peritoneal washings for staging determination of endometrial neoplasia. This position, which is based on data developed by the International Federation of Gynecology and Obstetrics (FIGO) in 2008, represents a departure from previous guidelines that incorporated pelvic washing cytology into staging schema. The AJCC maintains that the prognostic significance of peritoneal washings in this setting is "controversial" and that additional studies are needed. We sought to ascertain whether a positive pelvic washing cytology obtained at the time of tumor staging is of prognostic significance in patients with endometrial cancer.

Design: Patients diagnosed with endometrial cancer in the period from 1995 to 2009 at the study institution were identified utilizing available cancer center and pathology databases. The electronic medical record, pathology database, Cancer Center Tumor Registry, and State Registrar records were reviewed for tumor grade and stage at the time of surgery and clinical followup data with regards to recurrence. Two patient cohorts, based on staging designation according to the 1989 FIGO staging system, were selected and compared. Cohort 1 consisted of endometrial cancer patients with positive pelvic washings but, otherwise, with tumor confined to the uterine corpus (stage IIIA; T3a). Cohort 2 consisted of patients with stage I endometrial cancer (IA, IB and IC) with negative washings. The measured outcome was the presence or absence of recurrence. A minimum of one year follow-up was obtained for all patients.

Results: There were 30 and 72 patients within Cohorts 1 and 2, respectively. The latter cohort consisted of 24 stage IA patients, 36 stage IB patients, and 12 stage IC patients. Of the 30 patients within Cohort 1 (Stage IIIA), 3 patients had cancer recurrence. Of the 72 patients within Cohort 2, only 1 patient had cancer recurrence. The odds of disease recurrence for Cohort 1 was 7.88-fold higher than that for Cohort 2. The prognostic difference between cohorts was statistically significant ($p = 0.0412$).

Conclusions: Overall, irrespective of the findings on pelvic washing cytology, patients with endometrial cancer primarily involving the uterine corpus (Stage I disease; AJCC 7th ed) have a low likelihood of cancer recurrence. Nonetheless, patients with positive pelvic washings exhibit a significantly higher propensity for recurrence; hence, pelvic washing cytology is a relevant and important prognostic indicator.

402 Cytology-Histology Correlation of Mucinous Nonneoplastic Cyst of the Pancreas.

X Lin, R Nayar, B Zhu. Northwestern University, Chicago.

Background: A recently described mucinous nonneoplastic cyst (MNNC) of the pancreas is defined as cysts lined by mucinous epithelium and supported by hypocellular stroma without communication with pancreatic ducts. It is important to recognize MNNC since its management and prognosis are different from mucinous cystic neoplasm (MCN) and intraductal papillary mucinous neoplasm (IPMN). FNA cytomorphology of MNNC has not been described.

Design: 24 MNNCs diagnosed on surgical resection were retrieved. 18 had pre-surgery EUS-FNA biopsy. FNA cytomorphology and surgical histology were evaluated and correlated. Cyst fluid CEA and amylase concentrations were retrieved.

Results: FNA diagnoses included suspicious for adenocarcinoma (1 case), IPMN (1), suggestive of MCN (4), mucinous lesion (2), atypical (2), benign glandular cells (4), and unsatisfactory (4). FNA cytology showed flat honeycomb sheets/nests of cuboidal or columnar cells. Papillary architecture was seen in 2 cases, and abundant single cell pattern in 1. Goblet cells were sen in 18%. Cytoplasm was delicate or vacuolated (64%). Nuclei were round or oval and small to slightly enlarged with 1 or 2 inconspicuous nucleoli (prominent in 1 case), fine granular chromatin, and smooth nuclear contour (irregular in 1 case). Nuclear grooves and nuclear pseudoinclusons seen in 46% and 27%. Most cases showed watery mucin (thick mucin in 2). CEA ranged from 75.2 to 5,488 ng/ml and amylase from 19 to 28,478 U/L.

On surgical resection, MNNCs were randomly located in pancreas and were either unilocular or multilocular cysts lined by a single layer of bland columnar or cuboidal mucinous cells. Papillary structure was seen in 21% The glandular epithelial cells were diffusely positive for CK7 (100%), CD99 (basally, 100%) and PDX-1 (66%), focally positive for CD10 (superficial, 67%), CDX-2 (19%) and CK20 (6%), and negative for MUC2. Stromal cells in the cyst wall were focally, weakly positive for ER or PR (6%) and negative for inhibin.

Conclusions: MNNC shares clinical, radiologic and FNA cytology features with MCN and IPMN. Demonstration of communication with pancreatic ducts by radiology and IHC for CDX-2 and MUC2 (data not shown) are helpful to distinguish MNNC from IPMN. IHC for ER, PR and inhibin on cellblocks/needle cores is helpful to distinguish MNNC from MCN, however, IHC for MUC2, CDX-2, PDX-1, CK7, CK20, CD10, and CD99 is not useful (data not shown). Measurement of CEA and amylase is not useful. Combination of FNA cytology and IHC on cellblock/core biopsy along with clinical presentation and imaging studies is critical to diagnose MNNC on EUS-FNA and distinguish it from IPMN and MCN.

403 Indications for Kidney Fine Needle Aspiration (FNA) in the Era of Modern Renal Imaging Modalities.

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Background: Modern renal imaging modalities, particularly dedicated (thin-slice) renal CT scan with contrast and gadolinium-enhanced MRI, allow detailed evaluation of renal lesions, and the decision to follow-up or perform nephrectomy is often based on radiological studies alone without pathologic confirmation. Consequently, kidney FNA has become an uncommon procedure in our institution. The purpose of this study is to investigate the current indications for kidney FNA, assess its diagnostic utility, and evaluate cytologic/histologic correlation.

Design: All cases of kidney FNA performed in our center between January 2005 and August 2010 were retrieved. Clinical information, past medical history, radiologic findings, cytologic and subsequent histologic diagnoses, if available, were recorded.

Results: 41 cases (from 38 patients) of kidney FNA were identified: 23 (56%) were ultrasound-guided and 18 (44%) CT-guided. Indications for performing kidney FNA are summarized in table 1. The inconclusive solid masses were renal tumors with abnormal vascular pattern suggesting other entities in the differential diagnosis (oncocytoma, angiomyolipoma with minimal fat, urothelial carcinoma, or adrenal neoplasms).

Summary of clinical and radiologic indications for kidney FNA

Indications	Total number of cases
CT-scan favors renal cell carcinoma (RCC), but patient has prior history of other malignancy, extensive metastatic disease, or extensive local disease	17(41.46%)
Solid renal masses inconclusive on renal imaging	12(29.27%)
Abscess	4(9.75%)
Cystic lesions	3(7.32%)
Kidney masses on dialysis patients	3(7.32%)
Other (CT-scan favors lymphoma or urothelial carcinoma-1 case each)	2(4.88%)

In 29 cases (71%) a specific cytologic diagnosis was rendered. The nondiagnostic FNA samples (29%) consisted of foamy and hemosiderin-laden macrophages and normal renal elements. Histologic follow-up was available in 16 cases: 14 (87.5%) FNA diagnoses correlated with histology. Two cases (12.5%) were false negatives (FN): one FN case was Burkitt's lymphoma diagnosed on FNA as renal interstitial nephritis and the other was a clear cell RCC not sampled by FNA.

Conclusions: Kidney FNA represents a useful diagnostic tool in selected clinicoradiographic dilemmas. In our series the majority of FNAs (70.73%) were requested due to either the presence of confounding clinical factors with otherwise characteristic renal CT findings or inconclusive solid masses on renal imaging. The cytologic/histologic correlation is excellent (87.5%) with no false positive cases, however nondiagnostic samples are not uncommon.

404 What Is the Optimal Number of CINtec® PLUS Positive Cells for Detection of High Grade Cervical Intraepithelial Neoplasia?

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Background: p16 and ki67 have each been shown to be good biomarkers for high grade cervical intraepithelial neoplasia (HG CIN). Recently CINtec® PLUS (CINtec), a dual immunostain for p16 and ki67, has been proposed as a tool for the triage of women with atypical squamous cells of undetermined significance (ASC) and/or low grade squamous intraepithelial lesions (LSIL) on Pap smear. Based on the manufacturer's guidelines the presence of ≥1 CINtec positive cell is interpreted as a positive test result. This retrospective study was designed to determine whether increasing the threshold for a positive result improves the test utility for detecting an underlying or subsequent HG CIN.

Design: 152 cervical SurePath Pap smears (87 ASC and 65 LSIL) with histological and/or cytological follow up (range 1 to 83 mos; median 10 mos) were retrieved from our departmental files. The Pap stained slides were destained and then immunostained utilizing the CINtec®PLUS Kit (mtm laboratories, Inc Westborough, MA) that detects over-expression of p16 and ki67 as brown/cytoplasmic and red/nuclear reaction products, respectively. p16 or ki67 staining alone was recorded as negative. CINtec positive cells were counted in each smear and the cases were classified into four groups (A: 0 + cells, B: ≥1 + cell, C: ≥5 + cells, D: ≥10 + cells). Sensitivity, specificity, and positive and negative predictive values for detecting an underlying or subsequent HG CIN were calculated for each group.

Results: The correlation between the number of CINtec positive cells and detection of HG CIN in ASC and LSIL Pap smears is shown in the tables below.

ASC (N=87)

Number of CINtec + cells	Sensitivity	Specificity	PPV*	NPV*
≥ 1	62.5%	67.1%	16.1%	94.6%
≥ 5	50%	82.2%	22.2%	94.2%
≥ 10	37.5%	90.0%	27.3%	93.4%

LSIL (N=65)

Number of CINtec + cells	Sensitivity	Specificity	PPV*	NPV*
≥ 1	71.4%	47.1%	27.0%	85.7%
≥ 5	35.7%	66.7%	22.7%	79.1%
≥ 10	28.6%	84.3%	33.3%	81.1%

* PPV: positive predictive value, NPV: negative predictive value

Conclusions: Increasing the threshold number of CINtec® PLUS positive cells required for a positive test result increases the specificity of the test significantly without sacrificing the negative predictive value.

A prospective study is warranted to confirm our results.

405 Cytologic Features of Metastatic Papillary Thyroid Carcinoma in Cervical Lymph Nodes on ThinPrep Based FNA Preparations.

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Background: Liquid based cytology preparations (ThinPrep) in fine needle aspiration (FNA) have certain advantages when compared to conventional smears. The lesional cells are well represented on the ThinPrep slide for pathologic evaluation. But the cytologic appearance of FNA specimen on Thinprep slide is different from the conventional smear due to the differences of processing methods. FNA of neck lymph node to confirm metastatic papillary thyroid carcinoma (PTC) is one of the most common procedures in fine needle aspirations. The knowledge of the cytologic features of metastatic PTC on a Thinprep slide is very useful in routine practice. In this study, we reviewed 70 cases of metastatic PTC in cervical lymph node prepared by the Thinprep methodology. Cytologic features were systematically analyzed and compared to those from conventional smears and the FNA of the primary thyroid lesion.

Design: Thinprep FNA slides from 70 cases of metastatic PTC in cervical lymph nodes between 2003 and 2010 were selected. All the aspirations were satisfactory for diagnosis and have previously or concurrently sampled surgical specimens. The observations were focused on the cellularity, nuclear enlargement, nuclear grooves, intranuclear vacuoles, hypochromasia, colloid, and nuclear overlapping. All the cases are reviewed by three cytopathologists and each cytologic feature was graded as present or absent.

Results: As compared to the conventional smear, the Thinprep method provided a cleaner background, less blood contamination but fewer fragments of thick colloid plaques. Fifty cases had multifocal tumors in primary lesion. Most FNAs had moderate to high cellularity with fewer cases showing intranuclear vacuoles. The papillary structures were well preserved in the ThinPrep slides and nearly all cases showed nuclear overlapping and enlargment. The tumor follicular cells appear proportionally smaller than those on conventional smears.

Conclusions: Most cases (50/70) of metastatic PTC had a primary thyroid carcinoma with at least 2 tumor foci. This might be an indication to have lymph node metastasis of PTC. ThinPrep preparations contained satisfactory amount of representative lesional cells for pathologic evaluation which is comparable to conventional smears. The cytology appearances of PTC on ThinPrep are different from those obtained from conventional smear and it is important to acknowledge the differences in routine practice when using Thinprep to prepare the FNA specimen.

406 Outcomes in Thyroid Fine-Needle Aspiration with Atypical Follicular Cells: Risks of Malignancy and Integration into the Bethesda System for Reporting Thyroid Cytology.

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Background: The recently proposed Bethesda System (TBS) for reporting thyroid fine-needle aspiration (T-FNA) is a system intended to standardize terminology and offers specific cytologic categories to facilitate more useful reporting, data sharing and increased knowledge of the inherent risk of malignancy to optimize patient care. It has been suggested that the Bethesda category 'atypical follicular cells' actually encompasses four (4) subcategories that may have different risks of malignancy. In this study, we sought to determine whether or not there are significant differences in the risks of malignancy between the suggested subcategories of 'atypical follicular cells.'

Design: All ThinPrep® T-FNA between 2006 – 2008 at our institution with a cytologic diagnosis of 'atypical follicular cells' were retrieved and subclassified into one of four subcategories: 1) atypia, NOS; 2) atypia, rule out follicular neoplasm; 3) atypia, rule out Hurthle cell neoplasm; and 4) atypia, rule out papillary carcinoma. Histologic follow-up data were tabulated for each subcategory. The risks of malignancy were calculated and comparisons were made between the 'atypical follicular cells' subcategories. Categorical analysis was performed using a 2-tailed Chi-square test and a p-value of 0.05 was considered significant.

Results: A total of 2101 T-FNA cases were retrieved with 475 histologic follow-ups. A total of 186 (9%) were originally classified as 'atypical follicular cells,' and 164 (88%) had histologic correlation with an overall risk of malignancy of 23%. The risk of malignancy for atypical follicular cells subcategorized as 'atypia, rule out papillary carcinoma' was significantly higher (45%) than the other subcategories ($p = 0.026$). Cases subclassified as 'atypia, rule out follicular neoplasm' and 'atypia, rule out Hurthle cell neoplasm' did not have significantly different risks of malignancy ($p = 0.5$).

Conclusions: The subcategory 'atypia, rule out papillary carcinoma' has a significantly higher risk of malignancy when compared to the other atypical follicular cells. Cytologists should strive to communicate the risk of this subcategory to surgeons and clinicians when interpreting 'atypical follicular cells' in thyroid FNA.

407 Thyroid FNA Atypia of Undetermined Significance (AUS): Variability in Pathologist Reporting and Clinical Follow-Up.

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Background: It is suggested that AUS comprise approximately 7% of thyroid FNA results; however post-Thyroid Bethesda publications show a wide inter institutional variation in AUS percentages. We are a medical center with board certified cytopathologists and a large thyroid FNA volume. We have a high institutional AUS rate; however we have used a 6-tired system for reporting thyroid FNA since 2001 and included cases limited by qualitative factors and cystic lesions in AUS. Implementation of Bethesda criteria for thyroid reporting and transition from our previous reporting pattern was inconsistent between pathologists in 2008/2009, prior to the publication of the Bethesda atlas in December 2009. This review was undertaken to measure the variability between our cytopathologists with the aim of improving AUS reporting and its outcomes.

Design: We queried our institutional database for FNA biopsies categorized as AUS between 6/2008 and 12/2009. 399 thyroid biopsies were identified. We reviewed the cytopathology reports, along with repeat FNA and surgical pathology follow up data.

Results:

Pathologist Reporting and FNA/Surgical Pathology Follow Up

Pathologist	P1	P2	P3	P4	P5	P6	Total
Total Thyroid FNA	154	545	343	158	586	519	2305
AUS # cases	13	48	120	14	106	98	399
AUS Rate (%)	8.4	8.8	35	8.9	18.1	18.9	17.3
REASON FOR AUS							
% Hyperplasia vs. FN	54	73	62	36	64	64	64
% Quality	8	8	23	43	8	13	15
% CLT	31	8	9	14	10	14	12
% r/o Carcinoma/Lymphoma	8	2	3	7	18	3	7
% Cystic	0	8	4	0	0	5	4
REPEAT FNA* AFTER INITIAL AUS							
Negative %	67	33	65	33	42	48	53
AUS %	33	44	24	33	42	33	32
Neoplasm/Suspicious/Malignant %	0	22	4	33	17	19	12
SURGICAL OUTCOME							
Non Neoplastic %	67	69	85	50	50	32	54
Neoplastic (adenoma) %	0	25	15	25	29	49	31
Malignant %	33	6	0	25	21	19	15
Intradept. Consult %	15	21	8	0	6	2	7

* 3% of Repeat FNA were 'Unsatisfactory'

Conclusions: Our data confirms wide variation in the reporting of thyroid atypia among cytopathologists, with corresponding variable outcomes on repeat FNA and surgical follow up. We have identified specific trends for each pathologist. The results of this study will serve a useful quality assurance measure and be used for consensus building within our group. The use of intradepartmental consultation and review of specific thyroid Bethesda criteria for AUS, using representative case material from ones own practice are additional methods that may improve intra and inter institutional consensus in reporting thyroid FNA.

408 Endoscopic Ultrasound (EUS) Guided Evaluation of Upper GI Subepithelial Masses: Comparison of Jumbo Forceps Biopsies (JB) and Touch Preparation (TP) with Fine Needle Aspiration (FNA)/Cell Block (CB).

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Background: EUS-guided FNA is often performed for diagnosis of GI subepithelial masses. Alternatively, deep JB can be performed using JB forceps. JB is done via tunneling through the mucosa into the submucosa. A TP of JB allows immediate assessment of adequacy. The aim of our study was to compare the performance of EUS guided JB vs. EUS guided FNA in the diagnosis of subepithelial masses in the upper GI tract.

Design: We reviewed pathology reports and clinical information for 44 suspected upper GI subepithelial masses between 1/2006 and 6/2010. JB was performed with a Radial Jaw 4 forceps. FNA was performed with 19-25 gauge needles. On site evaluation (OSE) of adequacy was performed by a cytopathologist for all FNA and JB. Statistical differences of the two techniques were evaluated using Fisher's exact test.

Results: The location of masses was stomach (33), duodenum (8) and esophagus (3).

	No. of Cases	Adequate on "OSE"	CB	IHC on JB/CB	Definitive diagnosis
JB/TP	17	15 (88%)		15 (88%)	14 (82%)
FNA	14	10 (71%)	11 (80%)	6 (43%)	9 (64%)
JB/TP+FNA	13	11 (85%)	8 (62%)	10 (77%)	12 (92%)

There was no significant difference in the positive predictive value for OSE between the two techniques (FNA-only=90%, JB-only=87%, p=1). Three cases (JB=2, FNA=1) were non-diagnostic despite onsite "adequacy" due to misinterpretation of non lesional spindle cells. Final diagnostic adequacy was statistically higher using JB compared to FNA (90% vs. 67%, p = 0.03). The rate of diagnostic IHC using JB (88%) was greater than FNA/CB (43%, p = 0.018). There was no statistical difference in the proportion of definitive diagnosis obtained by JB+FNA/CB as compared to JB-only (p = 0.61).

Conclusions: JB with TP yields higher adequacy rates than FNA with CB for obtaining a definitive diagnosis by IHC. Concurrent FNA+JB during EUS did not provide any incremental diagnostic value compared to only JB with TP. Performance of OSE with TP for JB, provides the gastroenterologist with assurance that the "lesion" has been sampled and provides time and cost savings since concurrent FNA does not need to be performed. Availabilty of substantially more tissue by JB obviates the need for surgery if not indicated, and if surgery is performed IHC does not need to be repeated. For these reason, JB with TP has largely replaced FNA/CB for diagnosis of subepithelial masses by EUS in our clinical practice.

409 Endorectal Ultrasound Guided Fine Needle Aspiration (ERUS-FNA): A Diagnostic Tool for Immunohistochemical and Molecular Studies in Rectal and Perirectal Lesions.

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Background: Currently, the targeted therapy demands molecular study of the tumors in addition to an accurate morphological diagnosis. Colorectal endoscopic brushing and CT/ultrasound-guided FNA is commonly used in diagnosing and staging of colorectal and perirectal cancers. Endorectal ultrasound (ERUS) represents one of the most significant developments in endoscopy over the past 20 years. It allows highly detailed assessment of the gastrointestinal wall layers as well as visualization of the extraluminal structures. Furthermore, ERUS-FNA is gaining more clinical attention for obtaining material for molecular studies of tumors. Herein, we have reviewed the utility of ERUS-FNA in diagnosing of cancers and providing samples for immunohistochemical (IHC) and molecular studies in cancer patients.

Design: 47 perirectal FNAs were retrospectively retrieved from the cytopathology archives of a major academic medical center over a period of 20 years. 20 CT or ultrasound guided FNA cases were excluded. The cytomorphologic features of those 27 ERUS-FNA cases along with their corresponding histology were correlated with ancillary studies including IHC, and clinical information.

Results: The patients' age ranged from 17 to 79 years (mean=51.5 years), with a male to female ratio of 10:11. Among 27 ERUS- FNA cases, there were 3 cystic and 24 solid masses. The aspirated site included perirectal lymph nodes (n=7) and perirectal soft tissue (n=20). Metastatic carcinoma detected in 4 lymph nodes. Two lymph nodes were benign and one was non-diagnostic. Soft tissue FNAs showed carcinomas (n=9), benign glandular epithelium (n=9), one case diagnosed as cellular atypia and one was non-diagnostic. Past medical history of 18 cases was significant for carcinoma. The primary site of the tumors included colorectal, urinary bladder, prostate, pancreas, ovary and lower female genital tract. Multiple IHC stains were performed on cell block of 3 cases.

Conclusions: ERUS-FNA is a valuable diagnostic tool in diagnosing of perirectal lesions. ERUS-FNA can be utilized for 1) appropriately staging of cancer patients by evaluation of nodal metastasis or local tumor spread particularly in colorectal adenocarcionmas, 2) prevention of unnecessary surgical intervention in benign conditions, 3) providing diagnostic material for molecular and IHC studies.

410 Fine Needle Aspiration Biopsy Cytology of 42 Cases of Intraductal Papillary Mucinous Neoplasm.

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Background: Intraductal papillary mucinous neoplasm (IPMN) is often first sampled by endoscopic ultrasound guided fine needle aspiration biopsy (FNA). Early diagnosis of IPMN is advantageous because up to 35% of IPMN are associated with foci of adenocarcinoma. Previous studies offer conflicting results on the clinical utility of FNA in the evaluation of IPMN. Most series study fewer than 30 cases. We undertook a retrospective analysis of resection proven IPMN to determine which FNA findings are diagnostic of IPMN and whether the grade of dysplasia and presence of invasive carcinoma can be predicted.

Design: Resections of IPMN previously sampled by FNA within two years were retrieved from our database. Parameters assessed in FNA specimens included: quality of mucin, nuclear, cytologic, and overall atypia, group architecture, necrosis and inflammation. The resection specimens were separately reviewed and graded by currently accepted criteria. The FNA and histologic diagnoses were correlated to determine the features important in the cytologic evaluation of IPMN.

Results: 42 FNA specimens corresponded to 41 cases of IPMN. FNA diagnoses were adenocarcinoma (29%), mucinous cystic lesion (39%), acellular (19%), benign epithelium (21%), and low grade ductal lesion (2%). 26 IPMN (63%) and 15 IPMN associated with invasive adenocarcinoma (37%) were resected. Enteric type epithelium (50%) was most common with gastric (34%) and pancreatobilliary (11 %) types seen in the remainder. IPMNs classified as mild, moderate and severe dysplasia accounted for 28%, 36% and 26% respectively. FNA characteristics suggesting the diagnosis of IPMN included presence of mucin (86%), thick "colloid-like" mucin (55%), papillary groups (36%), and finger-like projections (31%). Nuclear enlargement, pleomorphism, cytoplasmic vacuolation, and nucleoli were included as atypia. Atypia was absent in 38%, mild in 10%, moderate in 7% and severe (adenocarcinoma) in 31% of FNA.

Conclusions: While the diagnosis of IPMN is suggested by FNA, definitive diagnosis of the neoplasm remains problematic, in part due to sampling issues. The presence of thick "colloid-like" mucin and delicate papillae are suggestive of the diagnosis on IPMN, but do not correlate with the grade of dysplasia or presence of malignancy. At the same time, FNA can be extremely specific in identifying severe atypia which is highly predictive of invasive carcinoma and may guide the surgical and medical management of the patient.

411 Performance of a Gene Expression Microarray Assay To Determine Tissue of Origin in Cytology Body Fluid Specimens.

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Background: Identification of the tissue of origin is a common challenge for cytology specimens. Cytologic body fluids are routinely obtained in the diagnostic workup of cases with a metastatic tumor of uncertain origin. The Pathwork Tissue of Origin (TOO) test was recently cleared by the FDA as an in vitro diagnostic device for formalin-fixed paraffin-embedded (FFPE) tissue. We evaluated the performance of this assay in body fluid specimens, including a comparison between specimens preserved with thrombin and Cellient™ cell block methodologies.

Design: 37 body fluid specimens (29 metastases-positive and 8 metastases-negative) were tested; 8 of these had both thrombin (T) and Cellient (C) cell blocks (Total n=45). RNA was extracted from 10 micron sections and gene expression assays were performed according to a standardized protocol (Pathwork Diagnostics, Redwood City, CA). A Tissue of Origin report was generated for each sample, and compared with that of the reference diagnosis. In addition, results between the thrombin and Cellient cell block methods were compared.

Results: 7 samples were excluded due to an estimated tumor content of <60% after Pathologist's review. From the remaining 38 samples, 95% achieved successful labeling/amplification. Only 2 samples failed array data quality verification. Therefore, 34 of 38 specimens (89%) successfully yielded test results. All metastases-negative cases but one, showed an expression profile that was most similar to lymphoma, in agreement with the predominant presence of inflammatory cells. TOO results for 16/20 specimens with malignant cells (80%, T/C duplicates not counted) were concordant with the reference diagnosis. Upon review of clinical history, one discordant case originally reported as breast was confirmed as an ovarian metastasis, improving the agreement with reference diagnosis to 85%. Thrombin and Cellient block results were concordant in all cases.

Conclusions: Our results show that it is possible to obtain gene expression profiles from FFPE body fluid specimens using the FFPE version of the Pathwork Tissue of Origin test, when samples meet the >60% tumor content criteria. These results demonstrate that gene expression profiling in body fluid cytology specimens has similar performance characteristics to those obtained in FFPE tissue samples. Pathologist interpretation is important for fluid specimens since negative samples will yield a lymphoma result that is reflective of an inflammatory infiltrate.

412 Fine-Needle Aspiration Cytology of Epithelioid Hemangioendothelioma: A Study of 12 Cases.

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Background: Epithelioid hemangioendothelioma (EHE) is a vascular tumor which may arise in lungs, soft tissue, liver and rarely in other anatomic locations. EHE may be difficult to distinguish from epithelial neoplasms, particularly adenocarcinoma, a difficulty compounded in fine-needle aspiration (FNA) specimens. Only isolated case reports of the features of EHE in FNA specimens have been described. We sought to study a series of EHE cases in order to identify characteristic features which might be useful in distinguishing them from other tumors.

Design: Patients with histologically-confirmed EHE who underwent FNA of their tumors between 1994 and 2010 were identified from the institutional database. All available cytologic slides were reviewed and scored where possible. The following features were examined: cellularity; cell arrangement/architecture; cell shape; nuclear location; nuclear grooves; intranuclear pseudoinclusions (INPIs); cytoplasmic appearance; cytoplasmic border; intracytoplasmic lumina (ICLs); presence of erythrocytes within ICLs; and background.

Results: Cytologic slides were available for review in 15 cases (9 female and 6 male patients). The tumors were situated in bone (n=5), soft tissue (n=5), liver (n=4) and lung (n=1). Three cases (2 in soft tissue and 1 in liver) contained insufficient cellular material for cytologic diagnosis. The smears in the remaining 12 cases showed variable cellularity. Architecturally, the smears demonstrated predominantly dispersed single cells along with occasional cohesive cell clusters. The cells were usually epithelioid in shape, with dense cytoplasm, well-defined cytoplasmic borders and eccentrically located nuclei. There was variable nuclear pleomorphism and nuclear grooves were identified in 11 (92%) cases. Occasional mitotic figures were present in 4 cases. At least occasional INPIs and ICLs were present in 11 (92%) cases, and rare erythrocytes were seen within ICLs in 5 cases. The background contained hemosiderin and siderophages in 5 cases.

Conclusions: Although the low power architectural pattern and individual cytologic features of EHE are not unique to these tumors, the combination of the following features: predominantly dispersed single cells with occasional cohesive cell clusters, epithelioid cytomorphology, dense cytoplasm with well-defined cytoplasmic borders, ICLs (± erythrocytes) and INPIs, in FNA samples should raise strong suspicion for EHE. They should prompt immunohistochemical evaluation using vascular markers and clinico-radiologic-pathologic correlation.

413 Correlation of "Follicular Lesion" with Final Histopathology of Thyroid Lesions and Comparison to "Follicular Lesion of Undetermined Significance".

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Background: In the Bethesda Thyroid Fine-Needle Aspiration (FNA) Classification System, the category of "atypia of undermined significance or follicular lesion of undetermined significance" is appropriate for aspirations with "architectural and/or nuclear atypia that is not sufficient to be classified as suspicious for malignancy or malignancy. A diagnosis of "follicular lesion of undetermined significance (FLUS)" is reserved for aspirates that demonstrate a predominantly microfollicular pattern with minimal or no colloid, sparsely cellular specimens with Hürthle cell predominance, and other indeterminant features. From 2001-2008, Barnes-Jewish Hospital (BJH) used the term "follicular lesion" to describe thyroid aspirates with similar criteria. The objective of this retrospective study was to correlate thyroid lesions with a diagnosis of "follicular lesion" on FNA with histopathology and compare the results to published FLUS estimates for risk of malignancy and usual management.

Design: The study comprises of 512 patients at BJH with FNA diagnosis of "follicular lesion" from 2001-2008. Initial FNA diagnoses with histologic correlation were evaluated. FNA diagnoses of follicular neoplasm and histologic diagnoses of incidental papillary cancer and papillary microcarcinoma, defined as lesions less than 10 mm in greatest diameter, were excluded from the study.

Results: 512 thyroid FNA biopsies were described as "follicular lesion." Histologic follow-up was available in 279/512 (54.5%). Of these 279 patients, 235 (84.2%) have benign results and 44 (15.8%) have malignant results. The distribution of histopathology findings are summarized in Table 1.

Diagnosis	N	%
Nodular hyperplasia	168	60.2
Follicular adenoma	60	21.5
Follicular variant of papillary carcinoma (FVPTC)	27	9.7
Papillary carcinoma	15	5.4
Follicular carcinoma	1	0.3
Other carcinoma (Insular carcinoma)	1	0.3
Parathyroid adenoma / Hashimoto's thyroiditis	7	2.5

Conclusions: The majority of "follicular lesion" was benign. As our "follicular lesion" showed similar diagnostic criteria to FLUS, these results support the estimated risk of malignancy and the NCI recommendation of repeat FNA for FLUS. While 15.8% of our "follicular lesion" was malignant on histopathology (NCI estimated risk of malignancy is about 5-15%). The most significant proportion of malignant diagnoses was papillary carcinoma, especially FVPTC. It could be a potential pitfall of repeat FNA for this group of patients due to a paucity or lack of well defined nuclear features of FVPTC.

414 Utility of "Low-Grade Squamous Intraepithelial Lesion, Cannot Exclude High-Grade Squamous Intraepithelial Lesion" (LSIL-H) Usage as a Quality Assurance (QA) Measure.

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Background: LSIL-H is an increasingly used, equivocal interpretive category in gynecologic cytology. Studies have found that for a given laboratory, the histologic follow-up of cervical intraepithelial neoplasia 2 or greater (CIN 2+) to be intermediate between that following a cytologic diagnosis of low-grade squamous intraepithelial lesion (LSIL) and high grade squamous intraepithelial lesion (HSIL). Monitoring individual cytopathologists' use of LSIL-H could be useful as a QA measure in determining if the category is under/over-utilized and if the individual cytopathologist's outcome falls within the laboratory average.

Design: Papanicolaou tests (SurePath) performed at our institution between 1/09 and 4/10 were retrieved. We reviewed the records of 10 cytopathologists (CP) to identify diagnoses of LSIL, LSIL-H, and HSIL for each individual as well as the rate of CIN 2+ in follow-up (F/U) histologic specimens for each category. For LSIL, a random subset of cases was reviewed. The rate of LSIL-H diagnoses relative to the overall usage of LSIL (LSIL + LSIL-H) was also examined for each cytopathologist.

Results: Our analysis revealed that utilization of LSIL-H relative to LSIL and HSIL as well as associated outcomes on follow-up histology vary among cytopathologists:

Utilization of LSIL-H relative to LSIL and HSIL with associated histologic outcomes for individual cytopathologists

CP	LSIL-H with available histology	% CIN2+ on F/U of LSIL-H	LSILwith available histology	% CIN2+ on F/U of LSIL	LSIL-H/ (LSIL + LSIL-H)	HSIL with available histology	% CIN2+ on F/U of HSIL
1	15	20.0	54	9.3	11.4	18	72.2
2	6	50.0	39	15.4	13.0	3	100.0
3	8	12.5	37	2.7	8.7	11	90.9
4	23	34.8	93	10.8	14.8	22	81.8
5	12	41.7	51	7.8	6.5	16	68.8
6	57	33.3	95	6.3	17.9	42	73.8
7	12	41.7	46	8.7	7.5	12	75.0
8	33	18.2	81	6.2	13.6	34	67.7
9	7	28.6	29	3.5	16.7	3	66.7
10	20	25.0	55	7.3	11.7	7	71.4
Overall	193	30.6	580	7.8	12.2	168	76.8

Conclusions: Studying the pattern of LSIL-H utilization with corresponding histologic outcome for individual cytopathologists can provide a useful measure of quality assurance within a pathology practice. While the overall rate of associated CIN 2+ on follow up for LSIL-H is intermediate between that of LSIL and HSIL, the outcomes for individual cytopathologists vary considerably. Interestingly, conservative usage of LSIL-H was not associated with a better cytologic-histologic correlation.

415 Cytological Analysis of Small Branch-Duct IPMNS Provides a More Accurate Risk Assessment for Malignancy Than Symptoms.

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Background: The Sendai guidelines for management of patients with clinically suspected branch-duct IPMN recommend surgical resection for small (< 30mm) cysts with either a dilated main pancreatic duct (MPD) > 6mm, a mural nodule (MN) or symptoms. Cytological evaluation is controversial and a risk factor only when "positive". Our historical experience of aspirating almost all pancreatic cysts has shown that cytology adds significant value to the preoperative management of these patients. We evaluated the accuracy of cytology relative to symptoms as a risk factor for surgical triage in a cohort of small branch-duct IPMN without evidence of a dilated MPD or MN.

Design: We retrospectively reviewed clinical, radiological and cytological data of 31 histologically confirmed small branch-duct IPMN of the pancreas without evidence of a dilated MPD or a MN and with adequate EUS-FNA fluid for evaluation. Clinical and radiological parameters were retrieved from medical records. Symptoms were recorded as present or absent. Cytology with high-grade atypical epithelial cells or malignancy (HGA) was considered true positive, and their absence as true negative for predicting histology of high-grade dysplasia or invasive carcinoma in resection specimens. Performance characteristics of sensitivity, specificity, negative predictive value (NPV), positive predictive value (PPV) and accuracy were calculated for clinical symptoms and cytology for appropriate surgical triage.

Results: There were 31 branch-duct cysts with a mean size of 18.7mm in 22 females and 9 males with an average age of 67 years; 55% were symptomatic, and 25.8% cyst fluids contained HGA. The table below compares the performance characteristics of surgical triage based on symptoms versus cytology with HGA.

Characteristic	Sensitivity	Specificity	PPV	NPV	Accuracy
Symptoms	.60	.46	.18	.86	.48
Cytology (HGA)	.80	.85	.50	.96	.84

Conclusions: Cytology was significantly more sensitive, specific and accurate than symptoms in predicting malignancy in small branch-duct IPMN without other risk factors. A triple negative test of negative dilated MPD, negative MN and negative cytology has a NPV of 96%, providing a much more accurate assessment for conservative clinical management.

416 Cytological and Immunohistochemical Features of EGFR and KRAS Positive Lung Adenocarcinomas: A Study of 51 Cases.

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Background: An association between mucinous bronchioloalveolar carcinoma (BAC) histologic features and EGFR and KRAS mutations has been reported in lung adenocarcinomas. However, many lung carcinomas are diagnosed using cytologic methods (FNA, cell block, touch imprint, needle core biopsy). Here we present the cytological heterogeneity and immunohistochemical features of lung adenocarcinomas and their relationship with EGFR and KRAS mutations

Design: We retrospectively reviewed the cytomorphological and immunohistochemical features of 51 lung cytology specimens that were previously tested for activating EGFR and KRAS mutations. Though BAC cannot reliably be diagnosed cytologically, features suspicious for BAC include bland mucinous epithelium, grooves and intranuclear pseudoinclusions. TTF-1 and cytokeratin immunostains were reviewed. DNA was extracted from archived paraffin-embedded cell block or frozen skinny needle core fragments. KRAS mutation analysis was performed on the 7500 Fast Real-Time PCR System (Applied Biosystems) using a set of 7 different TaqMan(r) allelic discrimination assays to detect 6 mutations in codon 12 (G12A, G12D, G12V, G12C, G12R, and G12S) and 1 mutation in codon 13 (G13D). Exon 19 deletions and the L858R mutation in exon 21 of EGFR were detected using PCR followed by capillary electrophoresis for fragment sizing on the CEQ 8000 platform (Beckman Coulter).

Results:

	EGFR+/KRAS-	EGFR-/KRAS+	EGFR+/KRAS+	EGFR-/KRAS-
n (%)	6 (12)	11 (21.5%)	1 (<1)	33 (64%)
Adenocarcinoma differentiation				
Well	3/6 (50%)	0	0	2/33 (6%)
Moderate	0	1/11 (9%)	0	9/33 (27%)
Poor	3/6 (50%)	7/11 (64%)	0	16/33 (48%)
NSCCa, poorly differentiated	0	3/11 (27%)	1 (100%)	6/33 (18%)
TTF-1	6/6 (100%)	11/11 (100%)	not done	22/31 (71%)
BAC- features	1/6 (17%)	0	0	3/33 (19%)
Mucin	2/6 (33%)	5/11 (45%)	0	15/33 (15%)

Conclusions: The majority of carcinomas with KRAS mutations were poorly differentiated (91%), whereas tumors with EGFR mutations were both well-differentiated (50%) and poorly differentiated (50%) adenocarcinomas. Mucin present in cases with KRAS mutations was predominantly in the form of intracytoplasmic lumina and/ or extracellular mucin associated with poorly differentiated cells. In previous studies, mucinous BAC- type differentiation correlated with the absence of EGFR mutation and presence of KRAS mutation; a finding that was not seen in this study. Further studies are warranted to correlate cytological findings with EGFR and KRAS mutations.

417 The Significance of Entosis ("Bird's Eyes") in Contemporary Urine Cytology Specimens.

AS Paintal, P Papas, A Contreras, RM Demay, W Reeves. University of Chicago Hospital and Medical Centers, IL; Baylor College of Medicine, Houston, TX.

Background: Urine cytology is useful in screening individuals for the presence of urothelial cell abnormalities and in follow up of patients with urothelial neoplasms. The presence of entosis ("bird's eyes") (BE) has been reported as a useful ancillary morpohologic finding which is correlated with urothelial neoplasia..

Previous reports have stated that, in the context of voided urine specimens, BE are relatively common in patients with urothelial carcinoma and relatively uncommon in patients no history of urothelial neoplasia.

Given the high frequency with which we see "bird's eyes" in urines in our practice, we were interested in exploring the relationship between BE and urothelial neoplasia.

Design: Randomly chosen cases of urinary cytology (voided urines, bladder washings, and catheterized urines) obtained between 2008 and 2010 were reviewed. All cases had histologic follow up within 6 months. Thin preps and cytospins in cases which were diagnosed as "atypical" or "suspicious" were reviewed by two pathologists under signout conditions for the presence of BE and this was correlated with histology on biopsy. In total, 69 specimens from 43 patients were evaluated. Histology on biopsy was divided into three categories: CIS/HG papillary carcinoma, LG papillary carcinoma, and benign with no history of urothelial neoplasia. In contrast to prior studies, the vast majority of our cases were bladder washes.

Results: We observed that BE were present in 20 of 29 "atypical" or "suspicious" urines (14/17 patients) with a concurrent histologic diagnosis of CIS/HG papillary carcinoma. BE were seen in 9 of 20 "atypical" or "suspicious" urines (6/14 patients) in cases of LG papillary carcinoma. Interestingly, BE were seen in 12 of 19 "atypical" or "suspicious" urines (9/12 patients) in cases with benign histology and no history of urothelial neoplasia. Of note, the majority of cases in this final group were diagnosed as "atypical" solely due to the presence of BE which ranged from focal to extensive. In contrast, in almost all "atypical" cases with BE and subsequent urothelial neoplasia, additional cytologic features of malignancy were identified. Bladder washes constituted the majority of specimens we examined.

Conclusions: BE are a common finding in the setting of urothelial neoplasia, but are not specific for malignancy in bladder wash specimens. The positive predictive value of BE as an isolated cytologic abnormality in bladder wash specimens is low.

418 Genotyping for HPV 16 and 18 in Women ≥50 Years of Age: Is It Useful?

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Background: Singnificant squamous cervical precursor lesions (CIN 2/3) and cervical squamous cell carcinoma are more commonly seen in women of reproductive age. However, due to a variety of hormonal and other factors, cytologic atypias are common in perimenopausal and postmenopausal women and may pose difficult management issues. Although HPV testing has been found to be useful in this patient population, the value of HPV genotyping, and in particular, testing for HPV16/18 is not well established.

The aim of this study was to identify the overall probability of having CIN 2/3 associated with HPV types 16 and 18 in women ≥50 years of age in comparison to women < 50 years of age.

Design: Our laboratory's records were searched for all women with liquid-based (Surepath) Pap tests (PT) with concomitant HPV genotyping data from January, 2001 – June, 2009. Women's age, cytologic diagnoses and HPV types identified were entered into a spreadsheet. The data was segregated into two major age groups, i.e. <50 and ≥50 years, and the follow-up biopsies obtained within 6 months of the Pap test diagnosed as CIN2/3 in both age groups were identified and further sub-classified into HPV 16/18 related and other high-risk HPV (31/33/35/39/45/51/52/56/58/59/68)-related CIN2/3.

Results: Of the 28,672 PT with HPV genotyping data identified during the study period, 5,418 PT were from women ≥50 years. 485 biopsies were done on hr-HPV+ women resulting in 30 diagnoses of CIN 2/3 in women with a PT diagnosis of NILM (n=1), ASC-US (n=17), LSIL (n=4), ASC-H (n=6) and HSIL (n=2). Of the 23,254 Pap smears performed in women under 50 years, 4,883 biopsies were done on hr-HPV+ women with 701 diagnoses of CIN 2/3. In the CIN 2/3 hr-HPV+ women under 50, 395 (56%) were positive for HPV types 16 or 18 while 306 (44%) were positive for other hr-HPV types. In the CIN 2/3 hr-HPV+ women over 50, 6 (20%) were positive for HPV types 16 or 18 while 24 (80%) were positive for other hr-HPV types.

	CIN 2/3 <50	CIN 2/3 ≥50
HPV 16/18	395	6
HPV, NON 16/18	306	24
Total	701	30

The overall increased probability of having CIN 2/3 in HPV 16 or 18 positive women compared to other hr-HPV types was 3.1 in women <50 and 2.7 in women ≥50.

Conclusions: Despite the fact that HPV 16/18 genotype was positive only in 21% of CIN 2/3 in women over 50 years of age, the relative risk of CIN2/3 in HPV 16/18+ women ≥50 as compared to that of other hr-HPV genotypes remains high. This supports the use of HPV 16/18 genotyping in this age group, although the percentage of HPV 16/18+ cases is small in this population in comparison to that of women <50.

419 Contribution of Bronchial Brushings to Diagnostic Yield of Superdimension® Navigational Bronchoscopy Procedures.

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Background: Electromagnetic navigation bronchoscopy (ENB) allows access to peripheral lung lesions that are beyond the reach of conventional bronchoscopes in a minimally invasive manner. Preliminary cytologic evaluation during these procedures is attempted to provide guidance for immediate management. After the navigational bronchoscope is introduced, brushes, aspiration needles, and forceps are introduced for specimen collection.

Design: Retrospective analysis of 32 cytopathology cases from 2007 to 2010 in which bronchial brushings (BB) were obtained using the superDimension® Electromagnetic Navigation Bronchoscopy system was performed to determine if evaluation of the BB contributed to the diagnostic yield of the case. Number of BB passes, concomitant endobronchial ultrasound guided fine needle aspiration (EBUS-FNA) and biopsy results, and clinical and pathologic followup were recorded. Slides were prepared by smearing the brush on a glass slide and staining with Diff-Quik stain and immediately examined without a coverslip. Rapid assessment of the BB and EBUS-FNA specimens was performed by cytopathology attending staff and fellows.

Results: Preliminary assessment was provided in 31 of 32 BB cases examined. Mean number of BB slides/case was 3.1. 25 cases had concomitant EBUS-FNA, and 22 cases had concomitant transbronchial biopsy (TBB). Of the 31 BB preliminaries, 5 had definitive diagnoses (4 for malignancy, 1 for fungus), 18 were negative, and 8 were atypical or suspicious for carcinoma. The five definitive cases were confirmed by biopsy, EBUS or clinical impression. Four atypical cases were diagnosed as cancer at final sign out or on a subsequent specimen, 1 was shown to be fungus, and the remaining 3 were negative. Followup of the 18 negative cases showed that 12 had tissue followup (8 TBB, 3 percutaneous core biopsy, 2 wedge biopsy, 1 subcarinal FNA). All of the followup TBB were negative. The 5 patients with wedges or cores were shown to have malignancies. 5 patients with negative BB with or without negative TBB were clinically felt to have malignancies and presumptively given radiation therapy.

Conclusions: Preliminary assessment of bronchial brushings from ENB procedures yielded definitive diagnoses in only 16% of cases (95% CI .03-.291) at the time of the procedure. Rapid assessment of BB specimens can be challenging and time consuming. Reactive bronchial cells may appear quite atypical when stained with Diff-Quik and examined without a coverslip. Definitive diagnosis at the time of the procedure should be reserved for straightforward cases, as this will usually abort the procedure.

420 Accuracy of Grading of Urothelial Carcinoma (UCA) on Urine Cytology: A Measure of Interobserver (INTEROA) and Intraobserver Agreement (INTRAOA).

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Background: The accuracy and reproducibility of histologic grading of UCA is well established however cytologic grading of UCA using current World Health Organization (WHO) criteria is not. We determined the accuracy and reproducibility of UCA grading in urine by measuring INTEROA and INTRAOA among 3 pathologists with and without expertise in cytopathology, compared with the gold standard biopsy or resection.

Design: Forty-four UCA-positive urines with tissue confirmation were blindly and independently graded twice (using WHO criteria) by 2 cytopathologists (MR, MS) and 1 genitourinary (GUP) pathologist (AO), with a 1-week-interval between grading. Pathologists were blinded to histologic findings. Coefficient kappa was used to measure INTEROA and INTRAOA. Accuracy was measured by percentage agreement with gold standard, for each pathologist on each occasion and for all pathologists and occasions combined.

Results: Histologic diagnoses included high-grade UCA (32/44, 73%), low-grade UCA (3/44, 7%) and carcinoma in-situ (CIS) (9/44, 20%). Grading accuracy ranged from 64%-89% for the 3 pathologists. AO was more accurate than MR (76% vs 60%) and MS (76% vs 67%) on both occasions combined. Overall accuracy for pathologists and occasions combined was 77% (95% C.I., 72% – 82%). INTEROA was unacceptably low [Table 1].

Coefficient Kappa for INTEROA

Observer	Occasion	Kappa	95% C.I.	p-Value
MS vs AO	1	0.05	-0.26, 0.36	1.000
MS vs MR	1	0.22	-0.08, 0.51	0.235
AO vs MR	1	0.37	0.09, 0.66	0.013*
MS vs AO	2	0.15	-0.19, 0.50	0.573
MS vs MR	2	0.57	0.30, 0.84	<0.001*
AO vs MR	2	0.29	-0.01, 0.59	0.053

* Significantly different from zero

Only 2 kappa values were significantly different from 0 and only one 95% C.I. included the value 0.75 (minimally acceptable for a reliable clinical measurement). No pathologist differed significantly in INTRAOA.

Conclusions: Although INTRAOA in cytologic grading of UCA was acceptable, INTEROA was unacceptably low. Additionally overall accuracy of grading was minimally acceptable at best. Because of unacceptable INTEROA, marginally acceptable INTRAOA and limited accuracy, cytologic grading of UCA is clinically unreliable and is not recommended for routine practice.The fact that GUP was the most precise suggests that accuracy is independent of expertise in cytopathology.

421 Improved Detection of Mucinous Neoplasms in Pancreas: Cytology and Histology Correlation.

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Background: Pancreatic cystic neoplasms include intraductal papillary mucinous neoplasm (IPMN), mucinous cystic neoplasm (MCN), serous cystadenoma (SCA), and pseudocyst (PC). Diagnostic tools include endoscopic ultrasound fine needle aspiration (FNA) cytology and cyst fluid analysis. Surgery is recommended in cysts with abnormal cytology or lesions >3cm with fluid CEA levels >192ng/ml since these likely are mucinous neoplasms (MN). Nonmucinous neoplasms (nMN) are characterized by lower fluid CEA concentration. Our aim was to correlate the cytologic findings with the fluid CEA levels and the surgical diagnosis.

Design: FNA of pancreatic cystic lesions and pancreatic surgical specimens during a 6-year period were reviewed. Surgical specimens were classified as MN and nMN and subcharacterized as IPMN, MCN, SCA and PC. FNA specimens were assessed for presence of thick mucin, neoplastic epithelial/atypical cells, and macrophages. CEA fluid values were collected.

Results: Out of 628 FNA performed, a follow-up surgery was available in 47 cases. Of these, 40 were MN and 7 were nMN. Histologically, 28 were IPMN, 12 MCN, 5 SCA and 2 PC. In the IPMN, cytology showed thick mucin in most of the cases (64%), neoplastic epithelial cells in 36%, atypia in 21% and macrophages in 29%. Cytology of most MCN revealed macrophages (92%) and thick mucin or atypia (3.8%). In the SCA, cytology showed rare epithelial cells, mucin and atypia (9%). In the PC, cytology revealed thick mucin in 2 cases. All the cases had variable amount of contaminant gastrointestinal epithelium. Sensitivity and accuracy of cytology for diagnosis of MN was 60% and 57%, respectively. CEA fluid levels were found in 28 cases. The mean for MN was 11501ng/ml and for nMN was 36.8ng/ml. When adding CEA fluid level, sensitivity and accuracy for diagnosis of MN increased to 83% and 79%, respectively. There were 6/40 malignant MN. Of these 4 showed malignant cytology and 2 were nondiagnostic. Sensitivity and specificity for diagnosis of carcinoma was 67% and 94%, respectively. Only 2 of these cases had fluid CEA analysis (34.2 and 140ng/ml).

Conclusions: Identification of thick mucin is a helpful cytological feature for diagnosis of MN, which should be distinguished from contaminant gastrointestinal mucus. The yield for diagnosing MN is poor but it is improved with the inclusion of CEA fluid level. Cytology detection rate for malignant MN is higher and fluid CEA level does not appear to increase the diagnostic yield.

422 Comparison of Urine Cytology and Fluorescence In Situ Hybridization in Upper Urothelial Tract Samples.

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Background: Fluorescence in situ hybridization (FISH) with the Urovysion® probe set (Abbot Molecular Inc., Des Plaines, IL) has been shown to detect chromosomal abnormalities in urine cytology with high sensitivity and specificity for bladder urothelial carcinoma (UC). Surveillance of the upper urinary tract (UT) urothelium remains challenging. The aim of this study is to compare the performance of cytology and FISH in the UT.

Design: UT urine samples (n=112) were collected from 61 patients (48 male, 13 female, mean age 66.2 years) via cysto/ureteroscopy from 2003-2009. Cytologic interpretation and FISH were performed using probes to chromosomes 3, 7, 17 and 9q21. Positive cytologic diagnoses were considered as positive and all other diagnoses were considered as negative. FISH was abnormal when identifying tetrasomy (≥10 cells with 3-4 copies of each probe (TETRA)) or hypertetrasomy/polysomy (≥4 cells with gains in at least two probes with one showing ≥5 signals (HT)). Biopsy diagnosis of UT cancer (n=21) within 2 years of the cytology/FISH result was the gold standard. In cases without a biopsy, a negative urogram or ureteroscopy with at least 1 year of follow up was considered negative (n=91). The mean follow up time was 3.2 years.

Results:

Upper Tract Specimens

	All cases		Cases with bladder UC excluded	
	Sens	Spec	Sens	Spec
Cytology	38% 8/21	89% 81/91	50% 7/14	95% 53/56
FISH HT	43% 9/21	84% 76/91	50% 7/14	89% 50/56
FISH HT & TETRA	67% 14/21	53% 48/91	79% 11/14	52% 29/56
FISH HT & Cytology	52% 11/21	77% 70/91	64% 9/14	84% 47/56

Cytology and FISH HT showed increased sensitivity over cytology alone (p=0.08), but had significantly decreased specificity (p=0.009). A majority of the false positives were caused by concomitant bladder UC. After excluding bladder UC, cytology and FISH HT had higher sensitivity (p=0.15) than cytology alone, but had significantly decreased specificity (p=0.01). Inclusion of TETRA as positive identified 3 additional cancers, but produced 28 additional false positives.

Conclusions: Compared to cytology alone, performing cytology with FISH on UT samples raises the sensitivity but significantly lowers the specificity. Specimens showing tetrasomy should not be reported as positive due to the high frequency of false positives. A large proportion of false positive results were likely due to bladder cancer contaminating the UT specimen during collection. We recommend that UT cytology and FISH should be interpreted with caution in patients with concomitant bladder cancer.

423 Atypical Squamous Cells of Undetermined Significance in Patients with HPV Positive DNA Testing and Correlation with Disease Progression by Age Group: An Institutional Experience.

EF Rodriguez, JP Reynolds, S Jenkins, MR Henry, A Nassar. Mayo Clinic, Rochester, MN.

Background: Atypical squamous cells of undeterminated significance (ASC-US) is a broad diagnostic category that could be attributed to human papillomavirus infection (HPV), malignant neoplasia and reactive conditions. The aim of our study is to evaluate our institutional experience with ASC-US diagnosis in patients with a positive HPV test, and identify the risk of progression in association with age.

Design: We queried our inhouse database (SNOMED) for all patients with a diagnosis of ASC-US and HPV positive results by the digene hybrid capture method from 2005 to 2009. We reviewed cytologic and follow-up surgical pathology reports for all specimens available. Progression was defined as a diagnosis of at least CINI on follow-up cytology, biopsy or resection.

Results: We identified 2613 patients. Follow-up was available in 1839, 346 of which had progression at 60 days and were excluded. A total of 1493 patients were included for time-to-progression analysis, 79.2% had one follow-up, 13.9% had 2 total follow-ups, 4.2% had 3 follow-ups, and the remaining had as many as 6 follow-ups. The total days of follow-up from baseline ranged from 5 to 1905 days (median 289 days; IQR=182 to 508). The number of days from baseline to the first follow-up ranged from 3 to 1711 days (median 239 days; IQR=156 to 395). Among the 1493 patients, 68.7% were age 30 or younger, 15.9% were between 31 to 40, 9.7% were between 41 to 50, and 5.6% were 51 or older. Disease progression within different age groups are illustrated on **Figure 1**. Approximately 30% of patients >40 years-old progressed versus 25% in older age groups (>50). Overall, there is not significant difference in disease progression between the different age groups (p = 0.29).

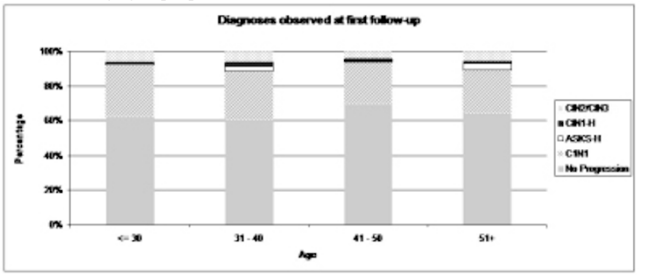

Figure 1: Pathologic progression by age

Conclusions: Although HPV is a risk factor for cervical cancer, only a minority (25-30%) progressed to dysplasia on subsequent follow-up in our study population. It appears that there is no difference in progression in the different age groups. Therefore, follow-up is warranted in women with HPV-positive ASC-US, regardless of age.

424 The Application and Diagnostic Utility of Immunocytochemistry on Direct Smears in the Subclassification of Non-Small Cell Lung Carcinoma.

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Background: Cytologic specimens are commonly obtained to establish a tissue diagnosis in patients with non-small cell lung cancer (NSCLC). Not uncommonly, though, cell blocks traditionally used for ancillary studies such as immunocytochemistry (ICC) are sparsely cellular or acellular. Given recent advances in chemotherapy for the treatment of specific subtypes of NSCLC, the importance of subclassifying NSCLC is becoming increasingly paramount. The two most common subtypes of NSCLC are adenocarcinoma (ADC) and squamous cell carcinoma (SQC). Recently, Napsin A expression has been reported in a high percentage of ADC but not SQC. Furthermore, a TTF-1(+) immunophenotype favors ADC over SQC. In contrast, strong p63 immunoreactivity favors SQC over ADC. In our ongoing study, we seek to investigate the diagnostic utility of ICC for Napsin A, TTF-1, and p63 on direct smears (DS) of NSCLC.

Design: First, the performance of Napsin A immunohistochemistry (IHC) was verified on a tissue microarray (TMA) composed of 117 lung ADCs. Next, ICC for Napsin A along with TTF-1 and p63 was performed on air-dried, unstained DS after brief formalin fixation and antigen retrieval in 15 cases. Surgically resected tumor tissue was available in nine of the 15 cases. In these cases, IHC for all three markers was performed, in parallel, using slides prepared from formalin-fixed, paraffin-embedded (FFPE) tissue blocks.

Results: First, the majority of lung ADCs on the TMA (85 of 117; 73%) were immunoreactive for Napsin A. Next, ICC for Napsin A, TTF-1, and p63 was applied to DS in 15 cases (10 ADCs and 5 SQCs). The results of the ICC along with IHC using FFPE tissue are summarized in Table 1.

Table 1. Results of ICC and IHC in DS of NSCLC

Case	Diagnosis	Napsin A (DS)	TTF-1 (DS)	p63 (DS)	Napsin A (FFPE)	TTF-1 (FFPE)	p63 (FFPE)
1	ADC	(-)	(-)	(-)	(-)	(-)	(-)
2	ADC	(+)	(+)	(-)	(+)	(+)	(-)
3	ADC	(+)	(+)	(-)	(+)	(+)	(-)
4	ADC	(+)	(+)	focal	(+)	(+)	focal
5	ADC	(+)	(+)	(-)	(+)	(+)	(-)
6	ADC	(+)	(+)	(-)	(+)	(+)	(-)
7	ADC	(+)	(+)	focal	(+)	(+)	focal
8	SQC	(-)	(-)	(+)	(-)	(-)	(+)
9	SQC	(-)	(-)	(+)	(-)	(-)	(+)
10	ADC	(+)	(+)	(-)			
11	ADC	(+)	(+)	(-)			
12	ADC	(+)	(+)	(-)			
13	SQC	(-)	(-)	(+)			
14	SQC	(-)	(-)	(+)			
15	SQC	(-)	(-)	(+)			

Conclusions: Our results, to date, provide promising evidence that direct smears represent a powerful resource for the application of Napsin A, TTF-1, and p63 ICC in the subclassification of NSCLC. Immunoreactivity for Napsin A and TTF-1 are often seen in ADC whereas a diagnosis of SQC is supported by a Napsin A(-)/TTF-1(-)/p63(+) immunophenotype.

425 The Application of Molecular Diagnostic Studies Interrogating EGFR and KRAS Mutations to Stained Cytologic Smears of Pulmonary Adenocarcinoma.

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Background: Cell blocks prepared from lung and mediastinal lymph node fine needle aspirates are routinely used for ancillary studies. Approximately 10% and 25% of pulmonary adenocarcinomas harbor mutations in epidermal growth factor receptor (EGFR) and KRAS, respectively. The most common EGFR mutations are in-frame deletions in exon 19 and the L858R substitution. KRAS mutations frequently involve codons 12, 13, and 61. Molecular diagnostic studies to interrogate these mutations are of increasing importance for gaining insight into prognosis and guiding the potential use of targeted chemotherapeutics. Unfortunately, in some cases, insufficient cellularity of cell blocks represents an impediment to the performance of these studies. Hence, we sought to investigate the utility of cellular material obtained from stained cytologic direct smears for EGFR and KRAS mutational analysis.

Design: Thirty-three cases of pulmonary adenocarcinoma (one air-dried, Diff-Quik stained direct smear per case) represented the source of material. Freshly collected and archived smears were used in 12 and 21 cases, respectively. Tumor cell-enriched areas from each smear were macrodissected for DNA isolation and purification. EGFR and KRAS mutational analysis was subsequently performed.

Results: The percentage of tumor cells in the extracted area on the direct smears ranged from 5 to 95% and exceeded 50% in the majority of cases (25 of 33; 76%). Sufficient yield of high quality DNA was obtained in all cases. EGFR and KRAS mutations were detected in three (9%) and 11 (33%) cases, respectively. EGFR and KRAS mutations were mutually exclusive.

EGFR and K-Ras Mutations Detected

EGFR mutation	# Cases
L858R	2
Deletion in exon 19	1
K-Ras mutation	# Cases
G12C	3
G12D	3
G12V	1
G12F	1
G13D	1
G12C & G13A	1
Q61H	1

Conclusions: Cytologic direct smears, routinely obtained during fine-needle aspirations, represent a valuable source of cellular material for molecular diagnostic studies and can be utilized in cases when a cell block exhibits insufficient cellularity or is not available. In our cohort of 33 cases of pulmonary adenocarcinoma, EGFR and KRAS mutations were detected at expected frequencies. Importantly, staining of the direct smears with Diff-Quik provides the opportunity for tumor cell enrichment by macrodissection and does not impair the ability to isolate high quality DNA for molecular studies. Archived or freshly collected cytologic smears are a viable, and in some cases, might be a preferred specimen source for molecular studies in pulmonary adenocarcinoma.

426 Utilization of Telecytopathology for Preliminary Diagnosis of Endoscopic Ultrasound (EUS)-Guided Fine Needle Aspiration (FNA) of Pancreas.

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Background: Onsite cytopathology interpretation is essential to improve the yield of EUS-guided FNA of pancreas. Distance and time constraints make onsite evaluation of EUS-guided pancreatic FNA time consuming. We evaluated a telepathology system for onsite-evaluation of EUS-guided pancreatic FNA.

Design: Real time images of Diff Quik stained cytology smears were obtained with an Olympus digital camera attached to an Olympus CX41 microscope. Cytopathologist accessing the dynamic images on a computer rendered preliminary diagnosis while communicating with the onsite operator over phone. Accuracy of preliminary diagnosis rendered via telepathology was compared with those procured by conventional on site assessment.

Results: A total of 110 EUS-guided-pancreatic FNAs were assessed for on-site preliminary diagnosis. Telecytopathology system and conventional microscopy were used to evaluate equal number of cases (55 each). Preliminary diagnoses of Negative/benign, atypical /suspicious and positive for malignancy were 69%, 7% and 24% for telecytopathology and 60%, 9% and 31% for conventional microscopy. The overall concordance between the preliminary and final diagnosis was 84% for telecytopathology and 87% for conventional microscopy. Of the nine discordant telecytopathology cases that were initially classified as benign, the final cytologic diagnosis included positive for malignancy (7 cases) and atypical cytology (2 cases). Conventional microscopy yielded 7 discordant cases with preliminary diagnosis of benign (5 cases) and atypical (2 cases). Final cytologic diagnosis on 5 benign cases included positive for malignancy (3 case) and atypical cytology (2 cases). Final cytology on 2 atypical cases was benign. Neuroendocrine neoplasms consisted of 31% of the total discrepant cases. Of the neuroendocrine neoplasms, telecytopathology missed 67% (4 of 6) of cases compared to (1 of 4) 25% missed on conventional microscopy.

Conclusions: Telecytopathology is comparable with conventional microscopy in accuracy of preliminary diagnosis during EUS-guided pancreatic FNA. Cytopathologist can make real time consultation from remote site and utilize time more efficiently. Neuroendocrine neoplasms prove to be diagnostically challenging for both telecytopathology and conventional microscopy. However the diagnostic accuracy for such cases is higher with conventional microscopy than telecytopathology.

427 KBA.62 and KIT Expression Patterns in Fine-Needle Aspirates of Malignant Melanoma Metastatic to the Liver.

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Background: KBA.62 has been described as a useful marker for diagnosing primary and metastatic malignant melanomas (MMM) in tissue. We stained fine-needle aspirates (FNA) of MMM to the liver for KBA.62 and compared it to standard melanoma markers, Mart-1 and HMB-45. Recent studies have shown that correlation between percentage of KIT-positive cells by IHC and *KIT* mutation status in MMM may be an important predictor of response to imatinib mesylate therapy. Since a large number of these metastatic tumors have been reported to express KIT, we also examined the liver FNAs for KIT expression.

Design: 22 cases of FNAs of MMM to the liver were retrieved from our files. Morphological examination of direct smears by Diff-Quik and Papanicolaou stains, as well as hematoxylin-eosin stained cell blocks was performed. Immunohistochemical (IHC) stains were evaluated using standard melanoma markers, Mart-1 and HMB-45, along with stains for KBA.62 and KIT. KIT expressing tumors were also evaluated for percentage of positive tumor cells. For cases negative for Mart-1 and HMB-45, additional IHC stains for S-100 and cytokeratin were performed.

Results: From the 22 cases reviewed, 21 (95.5%) stained positive for Mart-1 and 18 (81.8%) stained positive for HMB-45. 16 tumors (72.7%) stained positive for KBA.62 (strong membranous pattern), and 9 tumors (40.9%) stained positive for KIT (mostly showing cytoplasmic staining with membranous accentuation). 2 out of 9 cases showed KIT expression in >75% of tumor cells while 5 out of 9 cases had KIT expression in <25% of tumor cells. 4 cases that were negative for one or both melanoma markers

(Mart-1 and HMB-45) were strongly positive for S-100 and KBA.62 and negative for cytokeratin. The single metastatic ocular melanoma in the series was negative for KBA-62 but stained positive for KIT in >75% of tumor cells.
Conclusions: MMMs to the liver on FNA samples can be negative for either one or both commonly used melanoma markers. KBA.62 can be an equally effective melanoma marker for MMMs to the liver and may be used in cases that are negative for Mart-1 and HMB-45. KIT expression occurs in approximately 41% of MMMs to the liver, in keeping with previously reported studies.

428 High Sensitivity EGFR Mutation Detection in Cytologic Preparations Enabled by Laser Capture Microdissection (LCM).

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Background: The discovery of activating EGFR mutations in a subset of non-small cell lung carcinoma (NSCLC) was a major advance in our understanding of NSCLC biology, and has led to groundbreaking studies that have demonstrated the effectiveness of the tyrosine kinase inhibitors gefitinib and erlotinib in this disease. Fine needle aspirates (FNA) and other cytologic procedures have become increasingly popular for obtaining diagnostic material in NSCLC. However, frequently the small amount of material or sparseness of tumor cells obtained from cytologic preparations limit the number of specialized studies, such as EGFR mutation testing, that can be performed. In this study, we report a method, using laser capture microdissection (LCM), to assess EGFR mutations in cytologic preparations from small numbers of tumor cells.
Design: Cytology specimens including 3 FNAs and 2 pleural effusions from NSCLC patients with known EGFR mutations were reviewed. One case (lung FNA) containing an L858R mutation with sufficient material was selected to perform sensitivity assays. LCM was performed to obtain decreasing numbers of cells (250, 200, 150, 100, and 50) to determine the sensitivity of the method. 300-500 cells from the remaining 4 cases were obtained for study. DNA was extracted and divided directly into two reactions to detect the two most common EGFR mutations (exon 19 deletion and the L858R mutation), using capillary electrophoresis or pyrosequencing, respectively, after PCR.
Results: EGFR mutations were detected in all five cases, and were identical to the original mutations detected at the initial clinical evaluation. The sensitivity assay revealed that the L858R mutation could be from as few as 50 malignant cells. In one case, a pleural effusion, whole slide scraping (4 slides) of the cell block failed to detect the mutation; whereas LCM assisted analysis using a single slide (approximately 300 cells) was able to identify the appropriate mutation.
Conclusions: In this study we report a method that allows for detection of EGFR mutations in limited cytologic material using as few as 50 tumor cells. As more patients are diagnosed by minimally invasive procedures, such as FNAs and pleural taps, LCM can be a valuable tool to maximize the information that can be obtained from these small samples.

429 A Novel Technique for the Reduction of Unsatisfactory Rate Using the ThinPrep® Methodology.

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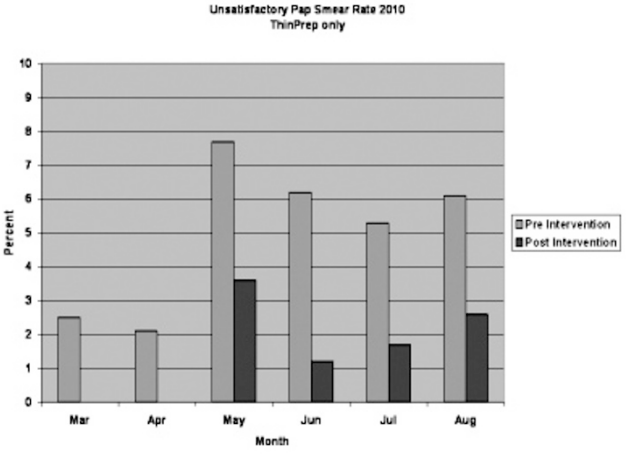
Background: Since the implementation of SurePath™ liquid-based methodology for Pap smears in our laboratory in 2004, the unsatisfactory rate has remained constant below 0.4%. A second liquid-based methodology (ThinPrep®) was introduced in January, 2010 to accommodate the outreach customer needs. Subsequent monitoring revealed an increase in the unsatisfactory rate. Root cause analysis showed this increase to be primarily due to the introduction of ThinPrep® methodology, possibly due to clogging of the pores in the filter of the ThinPrep® device by fibrin clot and excessive blood. Recommendations for overcoming these problems included treatment of bloody samples with glacial acetic acid prior to processing, to lyse the red blood cells. This method is labor-intensive. Recent studies have also raised the possibility of some changes in the cytomorphology of the squamous cells post-glacial acetic acid treatment.
Design: Development of a less labor-intensive and safe technique to maintain the unsatisfactory rate at or below the threshold of 1%. **Methods**: Due to low specimen numbers of ThinPrep® for the first four months our study was initiated from May 2010 to August 2010. Of 874 cases processed with ThinPrep® methodology, 80 (9.2%) smears were identified as unsatisfactory by cytotechnologists. The residual unsatisfactory specimen was washed using Thin Prep® CytoLyt® solution and then reprocessed on the ThinPrep® instrument. CytoLyt® solution is a commercially available methanol based, buffered solution used as a preservative to support cells during transporting specimen vials and slide preparation.
Results: After utilizing the reprocessing intervention 52/80 (65%) cases were interpreted as satisfactory. The ThinPrep® unsatisfactory rate before intervention was 6.4% which dropped down to 3% after intervention.



The combined (SurePath™ and ThinPrep®) unsatisfactory rate pre-intervention ranged from 0.8-1.4% while post-intervention rate now falls in the range of 0.4-0.6%.
Conclusions: This reprocessing technique is one the most successful attempts to reduce the ThinPrep® unsatisfactory rate. This intervention will reduce patient recalls for repeat collection avoiding delay in interpretations and identification of abnormalities.

430 Utility of Multiplex TTF-1 & Napsin A and p63 & CK5 Immunostains in Distinguishing Lung Adenocarcinoma from Squamous Cell Carcinoma in FNA Specimens.

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Background: The distinction of lung adenocarcinoma from squamous cell carcinoma has important therapeutic implications. Napsin A is a recently developed marker which has shown high specificity for lung tissue in surgical pathology specimens. In this study we evaluated whether use of novel multiplex TTF-1 & Napsin A and p63 & CK5 immunostains will improve the diagnostic accuracy of primary lung adenocarcinoma versus squamous cell carcinoma in FNA specimens.
Design: A total of 35 consecutive cases of formalin fixed, paraffin embedded adequately cellular FNA cell blocks, with a confirmed diagnosis of either lung primary adenocarcinoma (ACA, n=13) or squamous cell carcinoma (SCC, n=15) or poorly differentiated carcinoma (PDC, n=7) were included in the study. Two immunostain cocktails 'TTF-1 & Napsin A' and 'p63 & CK5' were used for dual immunostaining with cost analysis. All slides were scored in a blinded manner by two pathologists. The presence of one or more individual tumor cells with convincing brown nuclear TTF-1 and red cytoplasmic Napsin A immunostaining, cells with brown nuclear p63 and red cytoplasmic CK5 immunostaining, or cells with co-expression were interpreted as "positive".
Results: Good quality sections suitable for interpretation of immunostains were obtained from all 35 FNA cell blocks. All 15 FNA cell blocks from SCC cases were positive with the dual stain for p63 & CK5 and negative for the dual stain for TTF-1 & Naspin A. All 13 ACA cases were positive with the dual stain for TTF-1 & Naspin A and negative with the dual stain for p63 & CK5 (Sensitivity 100%; Specificity 100%). Of the 7 PDC cases, 5 cases were positive with the dual stain p63 & CK5 and negative for the dual stain TTF-1 & Naspin A consistent with SCC; 2 cases were positive with the dual stain for TTF-1 & Naspin A and negative for the dual stain p63 & CK5 consistent with ACA.
Conclusions: Primary ACA and SCC of the lung have distinct non-overlapping dual immunostaining patterns with Napsin A & TTF-1 in ACA and p63 & CK5 co-expression in SCC.
Napsin A & TTF-1 and p63 & CK5 dual immunostaining increases sensitivity and specificity of distinguishing primary lung ACA from SCC in FNA specimens.
The panel of multiplex Napsin A & TTF-1 and p63 & CK5 immunostains is potentially useful in differentiating ACA from SCC in lung FNA specimens with poorly diifferentiated carcinoma, especially in FNA specimens with scant cellularity.
The panel of multiplex Napsin A & TTF-1 and p63 & CK5 immunostains is cost-effective.

431 Cytologic and Molecular Insights into Urothelial Carcinoma Screening in Young Patients.

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Background: Urine cytology combined with Urovysion is the standard noninvasive method of screening for urothelial carcinoma. Development of urothelial carcinoma in patients less than 40 years of age is very rare; however, cytology and Urovysion is performed on these patients to evaluate various clinical symptoms and signs. There is scant literature on findings and utility of these screening tests in this population.
Design: A search of the PathNet clinical database was performed to identify urine cytology specimens collected between January 1998 and September 2009 from patients aged 18 to 40. The clinical history; indications for study; and biopsy, imaging, cystoscopy, and Urovysion results were collected for all cytology cases with an "Atypical/Inconclusive" diagnosis or worse.
Results: The search yielded 1191 cytology accessions from 837 patients. Seventy-five percent of patients had one specimen submitted for evaluation with the remainder

of patients having between two and eleven specimens submitted. The frequency of diagnoses were: Negative for Malignancy – 87%, Atypical/Inconclusive – 11%, Descriptive or Unsatisfactory – 1%. Eight cases were diagnosed as "Suspicious for Malignancy," and no cases were "Diagnostic of Malignancy." Seventy-five percent of patients with atypical cytology had the test performed for hematuria. Eight specimens were from patients with a history of a cancer diagnosis, including urothelial carcinoma, leiomyosarcoma, urachal carcinoma and colonic adenocarcinoma within a previous bladder reconstruction. Among specimens with atypical cytology, nephrolithiasis was the most frequently associated finding (27% of cases). Forty-four percent of patients had no cause for the hematuria or atypical cells discovered in their urologic work-up, with the majority of these undergoing both imaging and cystoscopy. All eight patients with suspicious cytology had a testing indication of hematuria. Similar to the atypical group, 25% had nephrolithiasis. All but one of these patients underwent cystoscopy with no lesions identified; one demonstrated inflammation of the bladder mucosa. Urovysion was performed on two suspicious cases and nineteen "Atypical/Inconclusive" cases. An abnormal result (loss of chromosome 9p21) was found in one of the cases with atypical cytology. However, repeat analysis was normal.

Conclusions: These results provide new insights into the cytologic and molecular findings in urine specimens from younger patients. This data may be useful in generating guidelines for the utilization of cytology and Urovysion in screening of patients with these demographics.

432 Prediction of Malignant Lesions by a Set of Four miRNAs in Thyroid Fine Needle Aspiration Specimens.

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Background: Cytological examination of thyroid nodules on fine needle aspiration (FNA) biopsies cannot reliably distinguish benign from malignant lesions for follicular patterned lesions. Using miRNA (miR) expression signatures as a diagnostic test is expected to improve the FNA diagnosis of thyroid nodules. The aim of this study was to further evaluate the diagnostic value of miRs in thyroid FNA pathology especially of the application of miRs in reclassification of indeterminate/suspicious thyroid FNAs.

Design: Seven miRs (miR-146b, -221, -187, -197, -346, -30d, -138) were selected; the expression levels were measured in archival thyroid FNA specimens.

Results: A set of 4 miRs (miR-146b, -221, -187, -30d) was identified which could differentiate benign from malignant lesions. The differential expression levels of the 4 miRs were analyzed by linear discriminant analysis (LDA), and a 4-miR LDA classifier was obtained to predict FNA specimens as benign or malignant and cross-validated the predictions by comparing with the histological diagnosis. For training sample set (n=60), we obtained diagnostic accuracy of 93.3%, sensitivity of 93.2%, and specificity of 93.8%. For validation sample set (n=42), we obtained diagnostic accuracy of 81.0%, sensitivity of 82.1%, and specificity of 78.6%. FNA cytology classified all the specimens into "indeterminate", "suspicious", or "malignant" categories. For the "malignant" FNAs, 45 out 47 (95.7%) were correctly predicted as malignant. For the "indeterminate" FNAs, 29 out of 33 (87.9%) were correctly predicted as benign or malignant. For the "suspicious" cases, 16 out of 22 (72.7%) were correctly predicted as benign or malignant.

Conclusions: Using miR expression profile, most of the thyroid FNA follicular lesions could be predicted as benign or malignant. This method could be potentially developed as a clinical assay in conjunction with FNA cytology to improve the accuracy and confidence of preoperative triage.

433 Reevaluating Utility of Bronchial Wash and Bronchioalveolar Lavage (BAL) at the Time of Transbronchial (Wang) Aspiration and Endobronchial Brushing Cytology.

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Background: Endobronchial cytology via transbronchial (Wang) fine needle aspiration and bronchial brushing are indispensable tools in the workup of patients with suspected pulmonary or thoracic disease. These procedures are well tolerated, cost effective, and less invasive than open surgical biopsies. The aim of this study was to evaluate the utility and diagnostic yield of bronchial brushing, transbronchial fine needle aspiration, endobronchial biopsy, bronchoalveolar lavage (BAL), and bronchial washing cytology in patients with suspected pulmonary or thoracic disease.

Design: In this study, we retrospectively evaluated cytology and surgical pathology reports from 168 total patients. These patients must have undergone bronchial brushing or endobronchial fine needle aspiration cytology. A total of 611 procedures were reviewed. These included the aforementioned as well as pleural fluid cytology, and cytology or tissue biopsy of other sites. The results were tabulated as positive for malignancy, positive for granulomatous inflammation, or negative. Diagnoses of "atypical" or "suspicious" were counted as negative.

Results: Of the 168 patients, a positive diagnosis was established in 118 with an endobronchial procedure. Of these, 12 were diagnosed with granulomatous disease, and 104 were diagnosed with malignancy. The yields were as follows: fine needle aspiration 75/214 (35.0%), bronchial brush 37/96 (38.5%), endobronchial tissue biopsy 33/95 (34.7%), bronchial wash 13/131 (9.9%), and BAL 2/44 (4.5%). Only 1 bronchial wash was positive in exclusivity, with a concomitant brushing of "suspicious". Zero BALs were positive in exclusivity. 26 patients required either tissue (18) or cytology (9) from an open thoracotomy (9) or another site for diagnosis. 24 patients had only negative findings and required no further workup.

Conclusions: Endobronchial fine needle aspiration and bronchial brushing cytology are invaluable diagnostic modalities in patients with suspected pulmonary or thoracic disease. Of the 168 patients in this study, 144 (85.7%) required no further workup and only 9 (5.3%) required an open biopsy. Bronchioalveolar lavage and bronchial washing proved to be of limited utility. At the time of bronchoscopy, additional material in the form of aspirations, brushings, or biopsies are of the highest yield. The cytopathologist can best serve the patient and conserve medical resources by discouraging BALs and washings in this setting.

434 Molecular Detection of *TOP2A* Gene Amplification in Archival Fine Needle Aspirates of Small Cell Lung Carcinoma.

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Background: Small cell lung carcinoma (SCLC) is a cytologic disease that does not lend itself to surgical resection because it is typically metastatic at the time of diagnosis. Despite ongoing advances in the molecular pathology of non-small cell lung carcinoma, many of which have resulted in improved targeted therapeutics, the molecular aberrations of SCLC remain poorly understood. Recent reports suggest promising therapeutic results with a new synthetic anthracycline that acts as a potent *topoisomerase IIα* (*TOP2A*) inhibitor. The primary aim of this study was to evaluate cytologic SCLC cases for *TOP2A* gene amplification to gain insight into the potential of *TOP2A* inhibitors to treat SCLC.

Design: A search for all cases of SCLC diagnosed via fine needle aspiration (FNA) at our institute over the past decade was performed. Cases with at least one slide that could be maintained for diagnostic records were assayed. An alcohol-fixed, Papanicolaou-stained slide from each case was decoverslipped and assessed by fluorescence *in* situ hybridization (FISH) using a ZytoLight® triple probe for centromere 17(C17), *human epidermal growth factor receptor 2 (HER2)*, and *TOP2A* (ZytoVision). Available corresponding formalin-fixed, paraffin-embedded (FFPE) samples from these tumors were also tested by triple probe FISH. The technique was optimized using decoverslipped, alcohol-fixed, Papanicolaou-stained cytologic smears of normal lung prepared from surgical bench specimens.

Results: FNAs from 37 patients and FFPE samples from 2 patients were evaluated. Of the FNA samples, 12 (32.4%) were disomic and 25 (67.6%) were aneusomic (trisomic up to sextasomic) for all three markers. Three specimens (8.1%) showed *TOP2A* amplification: two of these (5.4%) also showed *HER2* amplification (undetected in any other samples) and were disomic for C17, and the third was trisomic for C17 and *HER2*. Of the 2 correlate FFPE samples examined, FNA data matched in one instance and the other specimen was FFPE disomic and FNA aneusomic at the probe loci.

Conclusions: This FISH survey of SCLC FNA smears is the first of its kind and has resulted in the identification of three molecular profiles a single morphologic disease. Given recent reports on subsets of SCLC with improved response to a novel synthetic *TOP2A* inhibitor, it is of interest that this study identified a small subset of SCLC cases that had *TOP2A* amplification. This molecular heterogeneity may lend itself to more tailored therapeutics that are based not strictly on morphology but instead on the underlying molecular defect.

435 Timing of Repeat Thyroid FNA in the Management of Thyroid Nodules.

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Background: The Bethesda System for reporting thyroid FNA recommends repeat for an initial diagnosis of non-diagnostic or atypical cells/follicular lesion of undetermined significance. The interval recommended between the initial and repeat FNA for these patients is at least three months, however, there is limited data to support this recommendation. We investigated our own data to determine the yield of repeat FNA in relation to time interval between procedures and the original diagnosis.

Design: We retrospectively reviewed all reports on thyroid FNAs from 2006 to 2008 and identified those patients who had more than one FNA from the same lesion. Then the surgical pathology file was searched to determinine if any one had a thyroidectomy at our institution. All FNA diagnoses, time interval between procedures and the diagnoses of thyroidectomy were recorded.

Results:

Time Interval	Diagnosis of Repeat FNA (% of row total)				Total (% of column total)
	Non-Diagnostic	Sub-optimal Specimen	Atypical Diagnosis	Adequate Specimen	
< 2 weeks	4(14)	7(24)	2(6.9)	16(55)	29(9.4)
2 weeks-1 month	15(26)	15(26)	0	28(48)	58(19)
1-2 months	9(14)	18(29)	1(1.6)	35(56)	63(21)
2-3 months	5(12)	15(35)	1(2.3)	22(51)	43(14)
3-6 months	7(18)	9(24)	1(2.6)	21(55)	38(12)
6-12 months	4(11)	6(17)	4(11)	21(60)	35(11)
> 1 year	8(20)	11(27)	2(4.9)	20(49)	41(13)
Total (% of row total)	52(17)	81(26)	11(3.6)	163(53)	307

P = 0.43 by chi-square

307 patients met the criteria of the study and 81 had thyroidectomy. Overall 53% of the repeat FNAs yielded an adequate specimen for a diagnosis. This percentage did not vary significantly according to the time interval between procedures (see Table 1). However, those patients who had a non-diagnostic or suboptimal initial FNA were more likely to have a second non-diagnostic or suboptimal specimen than those whose first FNA was adequate (47% vs 29%, P = 0.03). Although limited by the small number, there was no evidence to suggest that a short interval was more likely to yield a false positive diagnosis on the repeat when compared to histology. Fifty-three percent (17/32) of patients who had at least one "atypical" diagnosis had surgery and 47% had malignancy. This percentage did not change significantly with the diagnosis of the patients' other FNA(s).

Conclusions: The diagnostic yield or accuracy of the repeat FNA is not related to the time interval between procedures but is related to the original diagnosis. Patients who had at least one "atypical" diagnosis and underwent surgery had a ~50% risk of finding malignancy regardless of the diagnosis of the patient's other FNA(s).

436 Age-Specific HPV, High-Risk HPV and HPV16/18 Rates and Follow-Up Histologic Diagnoses in Women with Atypical Squamous Cells of Undetermined Significance (ASC-US). A Study of 17,059 Cases from a Predominantly Low-Risk Screening Population.

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Background: ASC-US is the most frequent abnormal diagnosis made on Pap tests and the most frequent cytologic diagnosis leading to the diagnosis of clinically significant cervical precursor lesions (CIN 2/3).

The aim of this study was to review our experience with HPV reflex testing and genotyping and follow-up CIN2/3 diagnoses of women with ASC-US from a predominantly low-risk, suburban screening population

Design: All cases diagnosed as ASC-US according to the 2001 Bethesda System with a HPV test performed by PCR from 12/3/2003 to 6/30 2009 were entered into a spreadsheet together with the age of the patient, HPV type and follow-up biopsy diagnoses within 6 months. Any HPV+, high-risk HPV+ (HPV types included in the hc2 cocktail), HPV16/18+ and follow-up diagnoses of CIN2/3 were then compared.

Results:

Age	<20	20-29	30-39	40-49	50-59	>60	Number of cases of ASC-US (% of total ASC-US)
Total ASC-US	1182	5656	3702	3990	1784	745	17059
Any HPV type (% of all ASC-US)	720 (60.9%)	3054 (54.0%)	1303 (35.2%)	798 (20.0%)	427 (23.9%)	196 (26.3%)	6498 (38.1%)
HR-HPV (% of all ASC-US)	377 (31.9%)	1488 (26.3%)	594 (16.1%)	301 (7.5%)	118 (6.6%)	62 (8.3%)	2940 (17.2%)
HR-HPV (% of all HPV+ASC-US)	377 (52.4%)	1488 (48.7%)	594 (45.5%)	301 (37.7%)	118 (27.6%)	62 (31.6%)	
HPV16/18 (% of all ASC-US)	200 (16.9%)	836 (14.8%)	287 (7.8%)	122 (3.1%)	41 (2.3%)	24 (3.2%)	1510 (8.9%)
HPV 16/18 (% of all HPV+ASC-US)	200 (27.7%)	836 (27.3%)	287 (22.0%)	122 (15.2%)	41 (9.6%)	24 (12.24%)	
Biopsies (% biopsied)	242 (20.4%)	1391 (24.4%)	760 (20.5%)	564 (14.1%)	268 (15.0%)	93 (12.48%)	3318 (19.45%)
CIN2/3 (% of all ASC-US)	59(4.9%)	242(4.2%)	97(2.60%)	44(1.1%)	11(0.6%)	3 (0.4%)	456 (2.7%)
CIN 2/3 (% of all biopsies)	59(24.4%)	242(17.4%)	97(12.8%)	44(7.8%)	11(4.1%)	3 (3.2%)	
Risk of CIN2/3 in HPV16/18+ ASC-US	33 (42.9%)	129 (31.5%)	40 (26.3%)	13 (21.3%)	3 (12.0%)	1 (12.5%)	
Odds of CIN2/3 HPV16/18+ASC-US vs. non-16/18-HRHPV+ASC-US	4.0	3.5	3.4	4.1	4.0	4.1	

Conclusions: With increasing age of women with ASC-US, there is a steady decline in the percentage all HPV+, HR-HPV+ and HPV 16/18+ ASC-US, and CIN2/3 lesions. Genotyping for HPV16/18, however, still retains its value since across all age groups HPV16/18 have 4x more odds of CIN2/3 lesions on follow-up compared to all other HR-HPV.

437 Peripancreatic Paragangliomas: A Potential Diagnostic Pitfall in Cytopathology and Surgical Pathology.

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Background: Paragangliomas (PGs) or extra-adrenal pheochromocytomas are uncommon neoplasms arising in paraganglia and plexuses of the autonomic nervous system. In rare instances, PGs present around and involve the pancreas, mimicking a primary pancreatic lesion. We have collected a series of peripancreatic PGs clinically simulating a primary pancreatic lesion and discuss their clinical and pathologic features.

Design: Review of the cytopathology and surgical pathology archives at our institution between 1997 to 2010 identified 8 cases of peripancreatic PGs. Fine needle aspirates (FNA) and surgical pathology resections were reviewed for all cases. Demographic and follow-up information was also obtained.

Results: The cases consisted of 4 men and 4 women with an age range of 37 to 78 years (mean, 51 years). Patients presented clinically with either abdominal pain (5/8, 63%) or an incidental mass (3/8, 37%) discovered on routine radiographic imaging. All patients were found to have mass lesions suspicious for a primary pancreatic neoplasm on radiographic examination. The lesions were predominantly located in the body of the pancreas (5/8, 63%) and ranged in size from 5.1 to 17 cm (mean 10.9 cm). Five of 8 (63%) cases also demonstrated cystic change. FNA was performed in 5 cases. The diagnostic accuracy of FNA was low with 3 of 5 (60%) cases misdiagnosed as: pancreatic endocrine neoplasm (PEN) [n=1], retroperitoneal sarcoma [n=1] or pseudocyst [n=1]. In addition, 2 of 8 (25%) resection specimens were misdiagnosed as PEN. Immunohistochemistry was performed on all cases confirming the characteristic 2-cell populations. Follow-up information was available for all patients ranging from 2 months to 11.6 years (mean, 3 years). Two patients (25%) developed metastatic disease with multiple organ metastases and died 2.8 and 4.6 years after diagnosis.

Conclusions: Peripancreatic PGs are extremely rare tumors that may be mistaken for primary pancreatic lesions. In unsuspecting cases, the interpretation of FNA and surgical pathology resections can be diagnostically challenging. Awareness and proper recognition of this entity are imperative in ensuring proper patient management. Further, close follow-up of these cases should be considered due to the possibility of metastatic disease.

438 *BRAF* V600E Detection for Papillary Thyroid Carcinoma by LUNA ARMS PCR.

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Background: Detection of the BRAF V600E mutation in a thyroid fine needle aspirate (FNA) can provide important guidance for the preoperative diagnosis of papillary thyroid carcinoma (PTC), particularly for indeterminate samples. Existing assays may have a low sensitivity for the detection of BRAF-mutant tumor cells in a background of wild type/normal cells. A modified PCR technique, LUNA ARMS (Zhou, et al., 2010), was used to selectively block amplification of wild type BRAF with an unlabeled probe and ARMS primer that favor amplification/detection of the 1799 T>A mutation in exon 15, which changes Val to Glu. Mutation detection was by probe melting analysis.

Design: Patient samples with confirmed PTC or benign findings were selected. Tumor cell containing regions of formalin-fixed, paraffin-embedded (FFPE) sections and FNA slides were used for slide scrape and proteinase K DNA extraction. Paired FNA/FFPE samples, previously evaluated by a FRET PCR assay, were tested to determine if the increase in sensitivity of LUNA ARMS would resolve discordant cases. Additional cases with FNA, FFPE, and needle wash samples were also tested.

Results: An analytical sensitivitiy of <0.01% was obtained using dilutions of wild-type control and BRAF V600E mutation-containing cell line DNA. Forty-two cases with paired FNA and FFPE specimens were evaluated for the BRAF V600E mutation by LUNA ARMS PCR. Of 37 cases diagnosed histologically as PTC, 23 (62.2%) carried the BRAF mutation. For the FRET PCR assay, concordance of mutation analysis between FNA and FFPE samples was 100% for cases that were judged to be negative or PTC by cytology, whereas agreement was 72% for cases that were indeterminate by cytology. Concordance between FNA and FFPE indeterminate samples using the LUNA ARMS PCR test increased to >90%. Although the mutation may be detected in some cases in FNA needle washes, detection is more reliable from slide scrape lysates of FNA smears.

Conclusions: Because the surgical approach to patients with thyroid nodules is significantly guided by FNA biopsy, tests for PTC-associated mutations, such as BRAF V600E, can be a useful diagnostic adjunct. The presence of contaminating normal cells in FNA smears, however, requires that sensitive PCR tests be employed to detect the mutation. This study has shown that using the LUNA ARMS test results in the increased detection of BRAF V600E in FNA specimens.

439 Comparison between p16 Immunohistochemical Staining and HPV Testing in the Evaluation of Anal-Rectal Cytology.

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Background: p16 immunohistochemistry (IHC) can be utilized in the evaluation of cervical biopsies for high grade dysplasia. Studies have been done on cervical cytology Pap smears showing an increased specificity of p16 while maintaining a high sensitivity, compared to high risk HPV testing (Cancer Cytopathology 2010;118(3):146-156). Although there has been only 1 prior study correlating p16 with anal-rectal cytology results (Cancer Cytopathology 2006;108(1):12-21), we believe that this is the first study evaluating p16 IHC on liquid based, anal-rectal cytology and comparing the results with high risk HPV and biopsy results.

Design: Anal-rectal cytology specimens were retrospectively collected from 2005-2010. 178 ThinPrep (Hologic, Marlborough MA) specimens were diagnosed as satisfactory for evaluation from 138 patients and 139 (78%) had concomitant HR-HPV (Digene, Germantown MD) testing. Of the 178 specimens, 59 (33%) were NILM, 69 (38%) ASCUS, 3 (1%) ASC-H, 42 (24%) LGSIL and 7 (4%) HGSIL. Biopsy results were available for 25 patients (14%). The ThinPrep slides were decolorized and the p16 IHC (CINtec, Westborough MA) was performed and evaluated blindly without knowledge of the cytology, biopsy, and HPV results.

Results: Of the anal-rectal cytology samples with known HR-HPV results, 59% of the p16 results correlated with the HR-HPV findings, while 41% of the results were discordant (p16 or HR-HPV positive with the other one negative). 71% (5/7) biopsies diagnosed as AIN2 or 3 showed p16 positivity on cytology. Positive p16 staining was present in 86% of HGSIL, 55% of LGSIL, 23% of ASCUS and 5% of NILM. The 3 ASC-H specimens were all p16 negative. The main limitations of the p16 IHC were significant background debris staining, and positive staining of basal cells causing difficulty in differentiating from p16 positive high grade dysplasia due to dark staining and obscuring nuclear morphology.

Conclusions: p16 staining of anal-rectal cytology specimens correlated with the cytology results and HR-HPV findings. However, based on our experience we believe that the value of p16 IHC may not be as useful in the evaluation of anal-rectal cytology compared to results reported for cervical Pap smears. From this study, it appears that p16 IHC staining of monolayer smears do not show any advantage over concomitant HR-HPV testing.

440 The Utility of TLE1 as a Diagnostic Marker for Synovial Sarcoma Sampled by Fine Needle Aspiration.

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Background: Synovial sarcoma (SS) is an aggressive malignant soft tissue tumor that may also occur in visceral organs and mediastinum and is often evaluated by fine needle aspiration biopsy (FNAB). The presence of diverse histologic patterns of SS (biphasic, monophasic and poorly differentiated) can make diagnosing SS challenging, especially in small FNAB. Recent studies have shown that TLE1, a transcriptional co-repressor implicated in hematopoiesis, neuronal and terminal epithelial differentiation, is a promising diagnostic maker for SS in surgical specimens. In this study, we evaluated

the utility of TLE1 immunohistochemistry in distinguishing SS from its morphologic mimics in FNAB samples.
Design: In total, 9 FNAB cases of SS with cell blocks were evaluated, in addition to 25 cases of tumors in the differential diagnosis of SS: 5 thymomas, 8 peripheral nerve sheath tumors (PNST) (7 schwannomas and 1 malignant), 4 solitary fibrous tumors (SFT), 6 Ewing sarcomas (EWS), 1 dermatofibrosarcoma protuberans (DFSP) and 1 dedifferentiated liposarcoma (DDLPS). All diagnoses were confirmed by surgical resection and/or cytogenetic studies. Immunohistochemistry was performed on cell block sections using a polyclonal anti-TLE1 antibody. The extent of immunoreactivity was graded according to the percentage of tumor cells showing nuclear staining (>50%, 3+; 25-50%, 2+; <25%, 1+; no staining, 0) and the intensity of staining (strong, moderate, or weak).
Results: Strong nuclear expression of TLE1 was seen in 100% of SS cases, with 89% showing 3+ staining and all showing at least 2+ staining. Variable TLE1 staining was observed in 50% of PNSTs (12.5% 3+, 37.5% 1+ with 12.5% strong, 25% moderate, and 12.5% weak intensity). Weak to moderate TLE1 staining was seen in 75% of SFTs (25% 2+, 50% 1+ with 50% moderate and 25% weak intensity). Very limited TLE1 staining was observed in 20% of thymomas (1+, weak). EWS, DFSP and DDLPS were completely negative for TLE1.
Conclusions: TLE1 is a highly sensitive but not a completely specific marker for SS in FNA samples. Depending on the subtype of synovial sarcoma and the differential diagnosis, strong diffuse TLE1 nuclear expression can help distinguish SS from morphologic mimics such as thymoma, EWS and DFSP. However, caution must be taken for the interpretation of moderate TLE1 staining in samples containing a monomorphic spindle cell population when SFT or PNSTs are in the differential diagnosis. In such cases, molecular/cytogenetic confirmation of the SYT-SSX fusion gene is advisable.

441 A Novel Liquid Based ClearPrep Technique: Comparison to SurePath Methodology for Cervical Pap Smear.

LM Stoll, MW Johnson, S-L Yean, M Levin, J Rao. University of California, Los Angeles; University of Louisville, KY.
Background: Current liquid based preparations (LBP) including ThinPrep and SurePath have proven their efficacy compared to traditional smears for the diagnosis of cervical pathology. However, both methods rely on manufacturer-provided instrumentation and solutions for the preparation of specimens. We describe a new LBP, the ClearPrep method, which is a manual preparation, conducted using on-hand fixative and staining solutions.
Design: A total of 101 remnants of SurePath liquid-based patient samples were studied. These were consecutive samples collected from a period of 2 months (from June to July, 2010). The samples were divided in half by volume, one half for ClearPrep preparation and the other half for SurePath preparation. For ClearPrep preparation, the sample was vortexed for 10 seconds, followed by centrifugation (3000 rpm x 2 min). The specimen was decanted leaving the pellet and 1.5 mls supernatant. Three mls of polymer agar solution were added and the specimen vortexed (10 sec). The specimen was pipetted onto the slide, creating an oval shaped aliquot (1-1.5 mm). Slides were air-dried, stained with Papanicolaou method and cover-slipped. Both SurePath and ClearPrep slides were screened separately by both a cytotechnologist and pathologist.
Results: The age of women ranged from 20-74 years (median=38 yrs). Among the 101 cases, SurePath diagnoses consisted of: 6 unsatisfactory, 20 negative for lesion or malignancy (NILM), 12 NILM with endometrial cells, 26 ASCUS, 4 AGC, 25 LSIL, 4 ASC-H, 4 HSIL. ClearPrep slide diagnoses correlated in 97/101 (96%) cases. Discrepancies consisted of 4/101 (4%) ASCUS diagnosed on SurePath, with ClearPrep demonstrating NILM with reactive changes. One of these 4 cases showed positive HPV test.
Conclusions: This preliminary study shows that ClearPrep is comparable to SurePath in providing reliable diagnostic material for cervical Pap smears with potentially lower cost. Additional larger prospective studies are needed to determine the true cost-benefit of this novel method.

442 10-year Perspective on Cytomorphology, Cyst Fluid Carcinoembryonic Antigen (CEA), and Radiography for Endoscopic Ultrasound Fine Needle Aspiration (EUS-FNA) of Pancreatic Cystic Lesions.

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Background: EUS-FNA of pancreatic cysts is preferred pre-operative diagnostic method to differentiate mucinous from non-mucinous cystic lesions, as the latter doesn't necessarily require surgery. Our objective is to assess the utilities of cytomorphological examination, cyst fluid CEA level, and radiography in patient management.
Design: We investigated all EUS-FNA of pancreatic cystic lesions from 2000- 2010 and only cases with available surgical resection were included. Cytological features were reviewed for cellularity, presence of goblet cells, cell crowding, papillary architecture, nuclear atypia, and background of mucin. Cyst fluid CEA level and radiographic interpretation were collected. Based on the results of Brugge's study, a CEA level >=192 ng/ml was considered diagnostic for a mucinous cystic lesion.
Results: 32 patients were retrieved with mean age 65 (36-54), 13 Female. CEA was available for 16 patients with mean 756.9 ng/ml. 25 had radiographic evaluation. 25 patients had mucinous cystic lesions with 19 IPMNs and 6 mucinous cystic neoplasms; cytology correctly diagnosed 18/25 patients with high-yield cytologic features of presence of goblet cells, nuclear atypia, cell crowding, and papillary architecture; CEA correctly classified 10/13 patients with mean 1021.4 ng/ml; radiography correctly picked up 9/21 patients. Of the 7 patients with non-mucinous cystic lesions (5 serous cystadenomas, 2 pseudocysts), cytology correctly diagnosed 6 patients; CEA correctly classified 2/3 patients with mean 42.6ng/ml; radiography correctly assigned 3/4 patients. Cytomorphological examination had sensitivity 72%, specificity 85.7%, positive predictive value (PPV) 94.7%, negative predictive value (NPV) 85.7%. CEA had sensitivity 76.9%, specificity 66.7%, PPV 90.9%, NPV 66.7%. Radiography had sensitivity 42.9%, specificity 75%, PPV 90%, NPV 20%. When cytomorphological examination, CEA level, and radiographic evaluation were combined, 100% sensitivity and specificity were achieved.
Conclusions: 1). Neither radiography nor cyst fluid CEA alone is reliable for evaluating EUS-FNA of pancreatic cystic lesions; cytomorphological examination is still the golden standard preoperative diagnostic test. 2). All patients with mucinous cystic lesion had positive findings in one or more of three diagnostic tests; cytology diagnosis should be correlated closely with cyst fluid CEA level and radiographic findings. 3). Patients with negative findings in all three diagnostic tests can be safely observed.

443 "TelePAPology" vs Liquid-Based Thin-Layer Cervical Cytology: A Comparative Study Evaluating Specimen Adequacy and Non-Neoplastic Findings.

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Background: To date, the impact of digital imaging on routine day to day cytology remains far from perfect. Cellblock (CB) preparations from discarded/residual conventional and liquid based GYN samples have been shown to be of diagnostic value. In a pilot study, we have demonstrated the feasibility of utilizing imaging technology to overcome current limitations by digitizing cytologic specimens from CB preparations. The study was undertaken to evaluate the accuracy of TelePAP virtual slides from CB preparations in the determination of specimen adequacy and in the detection of various organisms and other non-neoplastic findings.
Design: The Cellient system from Hologic (Marlborough, MA) was used to prepare CBs. 231 H&E stained CB slides prepared from residual TP samples were analyzed. TelePAP slides were obtained using the Aperio digital imaging system (Vista, CA). They were reviewed by 4 cytopathologists and 2 cytotechnologists. Test performance characteristics of TP and TelePAP samples were compared for specimen adequacy, presence of organisms including bacterial vaginosis (BV), fungal organisms, trichomonas vaginalis (TV) and Herpes simplex, and non-neoplastic findings including reactive changes such as inflammation, radiation, glandular cells status post hysterectomy and atrophy.
Results: TelePAP virtual slides contained optimal amount of material from the overwhelming majority of cases. 7 cases were unsatisfactory due to absence of squamous cells. 19 cases were of suboptimal quality due to decreased cellularity (10 cases) or obscuring blood and/or inflammation. BV was diagnosed in 33 TelePAP cases as compared to 36 TP cases. Clue cells and filmy background of coccobacilli were evident. Budding yeasts and/or pseudohypheal forms were noted in 18 TelePAP vs 19 TP cases. TV organisms (10 cases) and one herpes case were identified in equal numbers of TelePAP and TP cases. Additionally classic radiation, inflammatory changes with reactive/reparative changes and endocervical and endometrial cells were easily identified in similar proportions by the 2 methods.
Conclusions: TelePAPology is a feasible method for widespread adoption to achieve high quality specimen preparations. The presence of organisms was identified on TelePAP slides as accurate as TP slides. TelePAP is as sensitive as TP method for detection of non-neoplastic findings. The cytologic/histologic findings are identical supporting the concept that this method is suitable for routine cytology, in situ and immunohistochemistry testing for HPV and other prognostic markers.

444 Application of BRAF V600E Mutation Detection to the Bethesda Thyroid FNA Classification System at an Academic Institution.

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Background: Thyroid Fine Needle Aspiration is the standard preoperative diagnostic procedure in the evaluation of thyroid nodules but suffers limitations. BRAF V600E positivity is associated with worsened clinical prognosis including lymph node positivity. Our purpose was to correlate BRAF mutational status based on PCR-SSCP technique in Thyroid FNA with surgical outcome and regional nodal status.
Design: 82 patients, 72% female (average age: 51 years, range 17-96) underwent BRAF PCR-SSCP testing as an adjunct test to the Bethesda Thyroid FNA Classification System at our institution beginning 2/2010. The analytical sensitivity of BRAF was previously determined to be <5%, suitable for cytological specimens.
Results: Of the 82 patients, BRAF V600E mutation was detected in 37, the remaining 45 were wild type. Pre-operative BRAF/Cytologic evaluation is summarized in Table 1.

BRAF Findings v.s. Cytology in Thyroid FNA

	Thyroid	FNA
Cytology Dx	BRAF (+)	BRAF (-)
Positive	32	7
Suspicious	3	9
Follicular Neoplasm	0	4
Atypical	5	32

55 patients underwent thyroidectomy, 34 also included regional lymph nodal dissection. BRAF positivity was associated with surgically confirmed lymph node metastasis in 59% of cases. BRAF negativity was associated with surgically confirmed lymph node metastasis in 41% of cases. BRAF, as a predictor had: sensitivity 61%, specificity 50%, positive predictive value 50%, and negative predictive value 59%.
Conclusions: Patients with BRAF mutation as determined by PCR-SSCP mutation testing in cytological specimens is more likely to found to have papillary thyroid carcinoma on resection. In addition, BRAF mutation testing offers a similar detection frequency in predicting surgical lymph node positivity as reported in the literature. Therefore, the pre-operative utilization of BRAF testing using cytological FNA specimen may offer a highly desirable preoperative guidance in the surgical treatment of papillary thyroid carcinoma.

445 Interobserver Agreement in Thyroid FNA (Fine Needle Aspiration) Diagnosis Using the New Thyroid Bethesda System Terminology (TBST) Classification: A Tertiary Care Community Hospital Experience.

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Background: There is limited literature evaluating the reproducibility and accuracy of the recently proposed TBST in particular the category of follicular lesion/atypical cells of undetermined significance(FLUS/AUS).We recently adopted TBST in our practice and the aim of this study was to evaluate the interobserver variabilility amongst multiple observers in the use of TBST.

Design: A study set of 60 cases of thyroid FNA with surgical pathology (SP) follow-up was created using 47 cases signed out as "cellular follicular lesion with atypia" or any cases flagged as "atypical" and 13 cases of hyperplastic nodule.6 observers (5 pathologists, 1 cytotechnologist, blinded to FNA and final tissue diagnosis) classified these cases according to TBST after a joint session of viewing text and images from TBST Atlas.SP follow-up included:26 cases hyperplastic/colloid nodule (HAN/CN), 24 cases follicular adenoma (FA) and 10 cases papillary thyroid carcinoma (PTC).Data was analyzed for paired interobserver agreement using Cohen's and multiobserver Fleiss' Kappa statistic.Accuracy of each rater was computed against the final SP diagnosis.

Results: Overall interobserver agreement across all TBST diagnostic categories was 0.26 amongst the 6 observers.Interobserver agreement(Cohen's Kappa)amongst pairs of 6 observers ranged from 0.16 to 0.69. Table 1 shows % cases of each observer classified as FLUS/ AUS and their follow-ups including the multiobserver Kappa agreement for each of the three diagnostic categories:

% of cases classified as FLUS/AUS by the 6 observers and surgical follow-up

	Path 1	Path 2	Path 3	Path 4	Path 5	Path 6	Kappa
Benign	28%	70%	47%	57%	48%	28%	0.32
FA	62%	30%	47%	14%	45%	56%	0.14
PTC	10%	0%	6%	29%	7%	16%	0.35

Table 1

Pairwise Cohen's Kappa for the 6 observers using 3 tier cytology against SP diagnoses were:0.40, 0.14, 0.33, 0.30, 0.28, and 0.41.

Conclusions: 1.Overall interobserver agreement in using TBST in classifying 60 cases amongst the 6 observers was fair. 2.Agreement among observers in the FLUS/ AUS diagnosis against the final SP diagnosis was poor to fair. 3.The correlation of individual TBST diagnosis and final SP follow-up was fair at best. 4.This suggests the need for additional refinement including joint sessions at the multi-headed microscope to improve agreement particularly in the category of FLUS/ AUS.5.Replication using a more extensive test sample and a random set of raters would be useful to quantify random variability in agreement for TBST classification.

446 Endocervical Adenocarcinoma *In Situ* and Invasive Adenocarcinoma: The Significance of HPV-DNA Results.

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Background: The association between adenocarcinoma *in situ* (AIS)/endocervical adenocarcinoma (ADCA) and human papillomavirus (HPV) is well established. The 2006 guidelines of the American Society for Colposcopy and Cervical Pathology recommend colposcopy and endocervical sampling for all cases of atypical glandular cells (AGC)/AIS. Previous studies have shown that significant pathologic lesions occur more frequently on follow-up biopsies in cases with AGC on cytology and positive HPV DNA testing, compared to cases with negative HPV testing. Outcomes of HPV testing, however, do not alter management of AGC. The aim of our investigation is to expand upon conclusions of prior studies regarding the sensitivity of HPV testing by selecting histologically confirmed AIS/ADCA and retrospectively assessing the results of HPV DNA testing.

Design: We searched archived material at our institution for cases of AIS/ADCA diagnosed on biopsy or hysterectomy over a five-year period. We then determined whether hybrid capture HPV DNA testing was performed, reviewed the cytologic diagnosis rendered on the corresponding specimen, and recorded the interval between HPV testing and biopsy/hysterectomy.

Results: We identified 3 and 22 cases of AIS and invasive ADCA, respectively. Of these 25 cases, 9 had corresponding HPV testing. Up to 3 HPV assays were performed per patient, yielding a total of 13 HPV assays. Of these, 9 were performed prior to the diagnosis of ADCA/AIS. Seven of these were positive, and 2 were negative. For the 7 HPV(+) cases, the corresponding cytologic diagnoses included 1 case each of ADCA, AGC, LSIL, and ASCUS, and 3 cases with no intraepithelial lesion. For the 2 HPV-negative cases, the respective cytologic diagnoses were ASCUS/AGC and no intraepithelial lesion. For the 7 HPV(+) cases, the interval between HPV testing and histologic diagnosis of carcinoma ranged from 18-353 days (average = 5 months). The period between HPV testing and histologic diagnosis was 331 days and 1145 days (average = 24 months) for the 2 HPV(-) cases.

Conclusions: Of 9 HPV assays corresponding to subsequently diagnosed cases of ADCA and AIS, 7 were positive (including all cases in which the interval between HPV testing and biopsy/hysterectomy was less than 11 months), and 2 were negative (including all cases in which the interval was greater than 11 months). These findings strengthen the association between HPV(+) results and presence of significant pathologic lesions such as AIS/ADCA, and provide further evidence supporting the relevance of HPV(+) results in terms of patient management.

447 Atypia of Undetermined Significance in Thyroid Fine-Needle Aspiration: Characterizing Cytopathologist Practice Patterns.

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Background: The standardized reporting framework established by The Bethesda System for Reporting Thyroid Cytopathology includes the category "atypia of undetermined significance" (AUS). This category should be used sparingly, with a proposed target rate near 7%. As the usage characteristics of this category are not well understood, the baseline AUS utilization rates over a five-year period were analyzed in anticipation of providing individual cytopathologist feedback.

Design: A customized report was written for the laboratory information system (LIS) that displays the category breakdown of thyroid fine needle aspiration (FNA) interpretations for the entire laboratory and for individual cytopathologists over a specified time period. With this report, a retrospective review of all thyroid FNAs from 1/05 to 12/09 was performed. All cases were reported using a six-tiered Bethesda-like diagnostic system. Results were compiled for individual cytopathologists, stratified by year, and correlated with histologic outcome when available.

Results: Seven cytopathologists evaluated a total of 5327 thyroid FNAs over this five-year period. An AUS diagnosis was rendered on 595 (11.2%) cases. Although the overall annual AUS rate for the laboratory remained relatively constant over this time period (range 9.9-12.4%), significant variability in AUS usage was seen not only between cytopathologists (individual averages: 6.1-18.7%) but also for individual cytopathologists over time (SD range: 1.4-4.0 percentage points). The AUS rate was unrelated to the experience of the cytopathologist. Correlation with histologic outcome data demonstrated an inverse relationship between the frequency of AUS and malignant outcome: the higher the AUS diagnosis rate, the lower the rate of malignancy (linear regression: R^2=0.46, $p<0.05$).

Conclusions: This study demonstrates that, in the absence of directed feedback, AUS usage is highly variable and often exceeds the recommended target of 7%. In addition, high AUS rates are not necessarily related to any inexperience on the part of the cytopathologist, and high AUS use is likely due to overcalling benign FNAs. Looking forward, the application of recently defined diagnostic criteria for AUS, along with periodic cytopathologist feedback, could help achieve a lower target for this category. Customized LIS reports displaying category breakdown of thyroid FNA interpretations for individual cytopathologists over a specified time period will likely be useful for this purpose.

448 Use of ThinPrep Imaging System for Primary Screening of Anal Paps: *A Pilot Study.*

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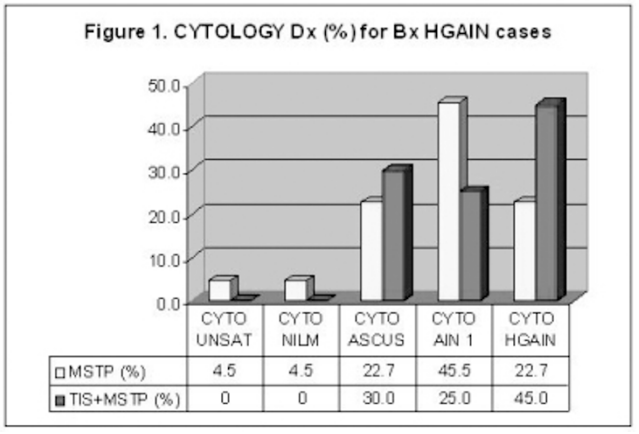
Background: Anal Pap (AP) is used for screening of high risk population and is similar in many respects to cervico-vaginal Pap (CVP). ThinPrep Imaging System (TIS, Hologic, Boxborough, MA) detects HSIL & Carcinoma in CVP with a sensitivity of 80% vs. 74% for manually-screened ThinPrep (MSTP) *(Biscotti. AJCP 2005;123:281)*. We sought to determine if TIS could also be utilized in primary screening of AP.

Design: AP from HIV positive patients were collected in PreservCyt® (Hologic, Boxborough, MA) via cytobrush without direct visualization and with sampling of transformation zone and processed as one Papanicolaou-stained TP. Bx of mucosal abnormalities were performed after acetic acid application via an anoscope. Two groups of AP were studied; 1) MSTP group: AP received from 01/09 to 05/09 and; 2) TIS+MSTP group: AP received from 01/10 to 08/10 (first screened on TIS and then manually screened). Results of both groups were correlated with biopsy (bx) obtained within 6 months. Technical details of TIS have been published (*(Biscotti. AJCP 2005;123:281)*.

Results: In MSTP group 45/292 cases from 44 patients [male, 34; female,10; age range, 21-61 years (yrs); mean, 43 yrs) had subsequent bx. Twenty-two of 45 cases (49%) had HGAIN on bx. Corresponding cytologic diagnoses were: HGAIN, 5 (23%); other < HGAIN, 15; Negative, 1; Unsatisfactory, 1 (Fig.1). Upon review, 5/17 false-negative (FN) cases were cytology undercall. In TIS+MSTP group 59/263 cases from 56 patients (male, 21; female, 35; age range, 26-69 yrs; mean, 47 yrs) had subsequent bx. Twenty of 59 cases (34%) had HGAIN on bx. Corresponding cytologic diagnoses were: HGAIN, 9 (45%) and other < HGAIN, 11 (55%). Upon review, 3/11 FN cases were cytology undercall and HGAIN cells were also present in the TIS 22 fields of vision (FOV). Sensitivity of AP for detecting bx-proven HGAIN was 23% and 45% for MSTP and TIS+MSTP groups respectively ($p=0.11$). Corresponding specificities were 91% and 79% ($p=0.20$).



Conclusions: 1) Primary TIS-assisted screening of AP increases sensitivity for detection of HGAIN *(p=0.11)*. 2) No bx-proven HGAIN cases were diagnosed as negative in TIS+MSTP group. 3) Three TIS+MSTP FN cases were interpretive errors as HGAIN cells were also present in the 22 TIS FOV.

449 Fascin as an Identifier of Metastatic Urothelial Carcinoma: A Retrospective Study of Fine Needle Aspirations.

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Background: Fascin immunohistochemical (IHC) staining has been shown to be a useful marker to determine invasion of urothelial carcinoma. Currently, thrombomodulin is the preferred marker for determination of primary site of metastatic disease in patients with concurrent or previously diagnosed urothelial carcinoma. Fascin, a marker of invasiveness, has not been correlated with metastatic disease. To enhance diagnostic accuracy and correctly identify primary site for appropriate patient management, fascin may be a useful marker in metastatic urothelial carcinoma.

Design: Twenty five cases with adequate cell block material for IHC staining were identified in surgical pathology and cytopathology files of metastatic urothelial carcinoma with either concurrent or previously resected urothelial carcinoma between 2005 and 2010. Fascin, thrombomodulin, uroplakin, cytokeratin 7, and cytokeratin 20 IHC were performed on paraffin-embedded cell block serial sections. Tissue microarrays with two 1mm cores of each of 26 renal and 46 prostate carcinomas were immunostained for fascin.

Results:

Marker Expression in Metastatic Urothelial Carcinoma

	Positive	Negative	Percent Positive
Fascin	23/25	2/25	92%
Thrombomodulin	20/25	5/25	80%
Uroplakin	0/25	25/25	0%
Cytokeratin 7	23/25	2/25	92%
Cytokeratin 20	7/25	18/25	28%

Fascin IHC Sensitivity and Specificity In GU Malignancies

Sensitivity	92%
Specificity	100%
PPV	100%
NPV	97%

Concordant results were seen in 22 of 25 (88%) cases for both fascin and thrombomodulin. Either fascin and/or thrombomodulin demonstrated positive staining in 25 of 25 (100%) cases. 100% of the 26 and 46 cases of kidney and prostate carcinomas respectively, were negative for fascin expression in the cells of interest.

Conclusions: Fascin is over-expressed in the majority (92%) of metastatic urothelial carcinomas. Previous studies have correlated fascin IHC staining with invasion. In comparing fascin with the traditional markers of urothelial carcinoma, fascin expression is of greater frequency and intensity than thrombomodulin. The combination of fascin and/or thrombomodulin identified all twenty-five (100%) cases of urothelial carcinoma. Fascin IHC staining is equivalent in frequency and intensity to cytokeratin 7. Fascin is an advantageous diagnostic complement to thrombomodulin and/or cytokeratin 7, in the setting of metastatic urothelial carcinoma, and is highly specific and sensitive relative to genitourinary malignancies.

450 Perivascular Mesenchymal Cell Clusters on Imprint Cytology of Lymph Nodes.

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Background: In stump cytology specimens of lymph nodes, other than lymphocytes, there are many mesenchymal cells, for example, tingible body macrophages, eosinophils, follicular dendritic cells, interdigitating cells, or fragments of capillary vessels, in the background. Furthermore, sometimes, large mesenchymal cell clusters are observed in the cytological specimen. The clusters are composed of smooth muscle actin-positive spindle cells and CD34-positive endothelial cells. We have termed these spindle cells Perivascular Mesenchymal Cell Clusters (PVMCCs). In this study, we investigated the clinical significance of PVMCCs in the imprint cytology of lymph nodes.

Design: Cases involved 144 imprint specimens of lymph nodes, 67 cases of B-cell lymphoma, 17 cases of T-cell lymphoma, 17 cases of Hodgkin lymphoma, 38 cases of reactive lymphoid hyperplasia, and 5 cases of Castleman disease.Two consecutive slides were prepared from each biopsy specimen. The slides were immediately fixed in 95% ethanol and stained using the standard Papanicolaou method. After cytomorphological evaluation, immunocytochemical staining was performed for SMA or CD34 on the destained Papanicolaou slides.

Results: In the core of the PVMCCs, CD34-positive spindle cells with ovoid nuclei and pale chromatin were seen. In some cases, the CD34-positive spindle cells were shaped like small, elongated vessels. Around the CD34-positive cells, there were many SMA-positive cells with poorly defined cytoplasmic boundaries, spindle-shaped nuclei, and dense chromatin. PVMCCs were found in 11 of the 67 cases of B-cell lymphoma, in 13 of the 17 cases of T-cell lymphoma, and 8 of the 17 cases of Hodgkin lymphoma. In particular, PVMCCs were found in 5 of the 6 cases of Angioimmunoblastic T-cell lymphoma. However, for non-tumorous lesions, capillary vessels are found in some cases, but PVMCCs were seen in only 3 of the 38 cases of reactive lymphoid hyperplasia. No PVMCCs was observed in the cases of Castleman disease.

Conclusions: PVMCCs were observed in 76% of T-cell lymphoma and 47% of Hodgkin lymphoma, but rarely observed in reactive lymphoid hyperplasia. (7%) It may be useful to differentiate between malignant T-cell lymphoma and reactive lymphoid hyperplasia.

451 FNAB of Secondary Neoplasms of the Thyroid Gland: A Multi-Institutional Study of 60 Cases.

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Background: Secondary neoplasms of the thyroid gland, either metastatic from a known primary site or from direct extension of a perithyroidal tumor, are rare. While a majority of secondary tumors can be readily diagnosed by fine needle aspiration biopsy (FNAB) based upon an associated clinical history and characteristic cytologic features, some secondary tumors can present a diagnostic challenge.

Design: We report one of the largest FNAB cohorts of 60 secondary thyroid neoplasms from 6 tertiary medical centers in the United States and Europe. All initial diagnoses were rendered by FNAB and correlate with clinical, cytologic, and ancillary study findings.

Results: Secondary thyroid tumors in our series were more frequent in women (n=35), and the average patient age was 59 years (range: 7-79 years). Based upon tumor type, squamous cell carcinoma (n=21) was the leading secondary thyroid neoplasm, followed by adenocarcinoma (n=12), renal cell carcinoma (n=7), melanoma (n=5), and non-Hodgkin lymphoma (n=4). Other rare secondary tumors in our series included adenoid cystic carcinoma, Hodgkin lymphoma, fibrosarcoma, liposarcoma, and leiomyosarcoma. Of the metastatic carcinomas, 20% originated in the kidney, 13% in the lung (3 squamous cell carcinomas and 5 adenocarcinomas), 8 % in the breast (5 cases) and 5% in the colon (3 cases). Eighty percent of the secondary neoplasms were accurately diagnosed by FNAB. Diagnostic difficulties occurred with adenoid cystic carcinoma and renal cell carcinoma because of its resemblance to primary oncocytic thyroid tumors. When material was available, immunohistochemical stains and flow cytometry were useful ancillary studies.

Conclusions: Secondary thyroid neoplasms are rare and can present diagnostic difficulties especially among tumors such as renal cell carcinoma which can mimic a primary thyroid tumor.

452 Fine-Needle Aspiartion Cytology of Breast with Prominent Lymphocytic Infiltrate: A Cyto-Histologyc Correlation Study.

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Background: The presence of prominent lymphocytic infiltrate in breast fine needle aspiration cytology (FNAC) brings up a differential diagnosis that includes benign and malignant lesions. Distinction among these processes is crucial because it dictates therapy and defines prognosis. The aim or our study is to review our experience with prominent lymphocytic infiltrate in breast FNAC in the diagnosis of benign and malignant lesions.

Design: Breast FNA cases performed at our institution between 1994 and 2010 were retrieved from the pathology electronic archives. Cytologic smears, cell block preparation, and histologic follow-up of lesions with prominent benign or atypical lymphocytic infiltrate were reviewed.

Results: Sixty one breast FNAC cases were selected, based on the presence of prominent lymphocytic infiltrate. Histologic correlation was available in 41 cases (67%). The patients age ranged from 22 to 99 years old (mean age: 56) and all were female. The aspirates were categorized as benign lymphoid cells in 24 cases (58%), reactive lymphoid cells in 2 cases (5%), atypical lymphoid cells suspicious for lymphoma in 8 cases (20%), and adenocarcinoma with prominent lymphocytic infiltrate suggestive of medullary carcinoma in 7 cases (17%). Overall, of the 41 cases with lymphoid cells, 14 cases (34%) had benign intramammary lymph nodes or fibrocystic disease, 8 (20%) had lymphoma, 16 (39%) had ductal or lobular breast carcinoma, and 3 (7%) had medullary carcinoma. Of the 24 cases with benign lymphoid cells, 12 (50%) had benign lymph nodes or fibrocytic disease, 1 (4%) had lymphoma, 9 (38%) had ductal or lobular carcinoma, and 2 (8%) had medullary carcinoma. Of the 2 cases with reactive lymphoid cells, 1 had lymphoma and 1 had invasive ductal carcinoma. Of the 8 cases suspicious lymphoma, 1 (13%) had fibrocytic change, 6 (75%) had lymphoma, and 1 (13%) had breast carcinoma. Of the 7 cases with lymphoid cells and atypical ductal cells suspicious for medullary carcinoma, 1 (14%) had fibrocystic change, 5 (72%) had invasive ductal or lobular breast carcinoma with prominent lymphocytic infiltrate, and 1 (14%) had medullary carcinoma.

Conclusions: The presence of prominent benign lymphocytic infiltrate in breast FNAC may be seen in different types of breast carcinomas, or may originate from an intramammary lymph node. Although rare, lymphoma is the most common diagnosis when atypical lymphoid cells are seen. A wide differential diagnosis including benign and malignant entities should always be considered when a prominent lymphocytic infiltrate is present in breast FNAC.

453 False Positive and Negative Rates of Pancreatic Endoscopic Ultrasound-Guided Cytologic Diagnosis: One Institution's Experience.

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Background: Primary diagnosis of pancreatic tumors is primarily based on endoscopic ultrasound (EUS) guided fine needle aspiration biopsy (FNA) and bile duct brushing (BDB). Diagnosis of malignancy is often complicated due to sampling difficulty and concomitant reactive changes associated with inflammation and atypia. Both false positive and false negative results lead to significant clinical consequences. For the purpose of quality control, we have reviewed our pancreatic cytology cases for the last six years.

Design: 733 pancreatic FNA biopsy cases including solid and cystic lesions were identified in our departmental files from 2004-2010. 264 pancreas resections were performed at our institution during this time period, of which 134 had prior cytologic diagnosis in our files (101 FNA and 33 BDB). Using resection diagnosis and clinical information, we compared both histological and cytological diagnoses to determine the false positive and false negative rate in our EUS-FNA diagnoses. All slides were independently reviewed by 3 pathologists (KW, TB, ZZ).

Results: 733 FNA cases were divided into 3 categories: positive or suspicious (290, 40%); negative or atypical (403, 55%) and unsatisfactory (40, 5%). Of the 134 cytologic cases (FNA and BDB) that had corresponding resections, there were 70 (52%) positive cases, 60 (45%) negative or atypical cases, and 4 (3%) unsatisfactory cases. Of those, 101 (75%) FNA cases included 58 (57%) positive, 39 (39%) negative, and 4 (4%) unsatisfactory diagnoses. There were 43 (32%) cases among the 134 cytology cases that had a diagnostic discrepancy (FNA, 20; BDB, 23). Among the 20 FNA cases, the majority of these represent a false negative diagnosis (19) with only one false positive case. The total false positive rate when including all positive FNA cases is 0.3% (1/290); while the total false negative rate is 5% (19/403). Among false negative FNA cases, only two cases were found to be negative due to interpretative error (2/403, 0.5%), while the remaining cases were negative due to sampling. Six cases (6/23) with interpretive error were found in the discrepant BDB cases.

Conclusions: EUS-FNA diagnosis for pancreatic lesions has very few false positives (0.3%) and false negatives (5%). The major cause of false negatives by FNA is due to sampling (17/19). Interpretative error of false negatives by cytopathologists is very low (0.5%). BDB was more likely to lead to a false interpretative error (BDB vs FNA, 6/23 vs 2/19) due to marked inflammation and the presence of ductal stents.

454 A Panel of CK7, CK20 and p63 Using the Cell-Transfer Technique Is Useful in the Fine-Needle Aspiration Diagnosis of Metastatic Urothelial Carcinoma.

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Background: Immunocytochemistry (ICC) performed on the cell-transferred fine-needle aspiration (FNA) smears is extremely helpful if conventional cell blocks lack adequate cellularity. Multiple immunostains can be performed on single cellular smear using the cell-transferred technique. Distinguishing high grade metastatic urothelial carcinoma (MUC) from non-keratinizing squamous cell carcinoma of the lung (NKSCCL) by cytomorphology alone can be difficult. The application of ICC on the cell-transferred smears is helpful to differentiate MUC from NKSCCL.

Design: A computerized search of our case file was performed for a 3-year period to identify all cases of MUC and NKSCCL with adequate materials for study. A panel of immunostains including CK7, CK20 and p63 was performed on the cell-transferred FNA smears as well as on sections from the corresponding original primary tumor. The concordant rate between the smears and the formalin-fixed tissue from the original tumors was calculated. Comparison of the immunostaining results of the MUC and NKSCCL cases was analyzed using the t-test.

Results: A total of 13 cases of MUC and 12 cases of NKSCCL were identified. The concordant rate of the immunostaining performed on the cell-transferred FNA smears and the formalin-fixed original tumor tissue was 100% for CK7 (25/25), 100% for p63 (25/25) and 92% for CK20 (23/25). All tumors (100%) including both MUC (13/13) and NKSCCL (12/12) were positive for p63. The majority of MUC cases (92%) stained with CK7 (12/13) with 11 of 12 demonstrating diffuse intense cytoplasmic staining; in comparison only 2/12 (17%) of NKSCCL showed focal or weakly staining with CK7. Five of 13 (38%) MUC and none of the 12 (0%) NKSCCL were positive for CK20. Diffuse strongly cytoplasmic staining with CK7 and/or positive CK20 staining favors the diagnosis of MUC over NKSCCL ($p < 0.01$).

Conclusions: ICC performed on cell-transferred cytologic specimen is very accurate with a high concordance rate comparable to the results obtained on the formalin-fixed tissue. A panel of CK7, CK20 and p63 performed on the cell-transferred smears is useful for the FNA diagnosis of metastatic urothelial carcinoma especially in cases lacking an adequate cell block. P63 is a sensitive marker that is positive in both urothelial and squamous carcinoma. In the appropriate clinical setting, the findings of intense CK7 staining and/or positive CK20 coexpressed with p63 is diagnostic of a metastatic urothelial carcinoma.

455 Expression of NeuroD and CD44 in the Fine Needle Aspirates of Pancreatic Endocrine Neoplasms.

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Background: Endoscopic ultrasound-guided fine needle aspiration (EUS-FNA) has been increasingly used in the preoperative diagnosis of pancreatic endocrine neoplasm (PEN). However, it is almost impossible to further classify PENs based on cytomorphologic features alone. There are also no validated markers that can be used for ancillary tests. NeuroD, a member of basic helix-loop-helix transcription factors, may be involved in neural development and differentiation. CD44, a membrane glycoprotein, has been shown to be associated with aggressive behavior, metastasis and poor prognosis in a variety of human tumors. This study is to evaluate expression of NeuroD and CD44 and explore their potential prognostic values in the fine needle aspirates of PENs.

Design: A total of 29 cases of PENs diagnosed by EUS-FNA were retrieved from cytopathology archives at our institution. Surgical follow-ups were available in 14 cases, including 9 cases of well-differentiated endocrine tumor/carcinoma and 5 cases of poorly differentiated/malignant endocrine carcinoma. NeuroD and CD44 immunostains were performed on the cell-block sections. Expression of NeuroD and CD44 were analyzed as percentiles of positive/total tumor cells, which were further compared between well-differentiated and malignant tumor groups.

Results: NeuroD expression with a nuclear staining pattern was seen in all cases of PENs. However, expression of NeuroD was significantly higher in the well-differentiated tumor group (ranging from 75% to 90%) as compared to the malignant group (ranging from 40% to 50%) ($p < 0.01$). CD44 expression with a membranous staining pattern was seen focally in all PENs (ranging from 5% to 20%) except for one case. Interestingly, the tumor with high CD44 expression (80%) was the only case that had a confirmed distant metastasis (liver).

Conclusions: This study demonstrates that NeuroD is differentially expressed in well-differentiated and malignant PENs and high CD44 expression appears to be associated with a more aggressive behavior, suggesting that NeuroD and CD44 can be potentially used as prognostic markers in the fine needle aspirates of PENs.

456 Utility of Immunostains in Differentiating Gastric-Type Pancreatic Intraductal Papillary Mucinous Neoplasms (G-IPMNs) from Gastric and Duodenal Mucosal Contaminants in Pancreatic Endoscopic Ultrasound-Guided Fine Needle Aspirates.

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Background: Due to their frequency and potential curability, IPMNs are a major target of pancreatic EUS-FNA. Their cytologic diagnosis may be difficult due to lack of cytologic atypia and resemblance to normal gastric (GM) or duodenal (DM) mucosal fragments, which are often contaminants of transgastric or transduodenal EUS-FNA. This study aimed to determine if immunohistochemical (IHC) stains may be useful to differentiate gastric-type IPMN (G-IPMN) from GM and DM.

Design: Tissue microarrays (TMAs) were constructed from 19 cases of resected G-IPMN, 20 GM, 20 DM and 20 benign pancreatic tissues. IHC stains AMACR (P504S), B72.3, CDX2, CK7, CK20, HepPar1, mCEA, MOC31, MUC1, p16 and villin were applied to TMA sections. The extent and intensity of staining were assessed on a 0-3+ scale for all stains, and digitized IHC staining intensity was determined for selected stains.

Results:

	Gastric mucosa (n=20)	Benign pancreatic tissue (n=20)	Duodenal mucosa (n=20)	IPMN Gastric type (n=19)
AMACR (P504s)	80%)	90%	100%	89%
B72.3	40%	0	5%	77%
CDX-2	5%	0	95%	10%
mCEA	65%	25%	50%	100%
CK7	70%	100%	15%	100%
CK20	70%	0	100%	42%
HepPar1	5% F	0	100%	32%
MOC-31	90%	100%	100%	100%
MUC1	88% (n=16)	91% (n=11)	0 (n=9)	30% (n=10)
P16	5% F	95% F	0	89%
Villin	55%	70%	100%	89%

P16 showed the best discrimination of GM from GT-IPMN: p16 stained nuclei and cytoplasm in 17 of 19 G-IPMN cases (89%), while only one GM with intestinal metaplasia was focally p16 positive. Other useful markers included villin, which showed a predominantly brush border pattern in G-IPMN (63%) versus a mixed brush-border/cytoplasmic pattern in 45% of GM, and 3.3-fold higher expression in DM versus G-IPMN ($p<0.0001$); and mCEA, which was diffusely positive in all G-IPMN but only weakly positive in 65% of GM, and 3.4-fold higher expression in G-IPMN than DM ($p=0.003$). Markers of limited utility included HepPar-1, which stained all DM samples but only 1 of 20 GM samples and 32% of G-IPMNs; and B72.3, which stained 77% of G-IPMNs and 40% of GM.

Conclusions: Our results indicate that p16, villin and mCEA may be useful in distinguishing G-IPMN from GM and DM contaminants in cell block sections from pancreatic EUS-FNA.

457 Utility of UroVysion and Cytology in Detecting Bladder Cancers: A Study of 1,835 Paired Urine Samples with Clinical and Histological Correlation.

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Background: Urine cytology has been widely used for screening of bladder cancer but is limited by its low sensitivity. UroVysion is an FDA approved molecular test that uses fluorescence in situ hybridization to detect four chromosomal abnormalities commonly occurring in bladder cancers. The present study evaluates the utility of UroVysion in detecting urothelial cell carcinoma (UCC) by correlating it with concurrent urine cytology, histological and cystoscopic findings, and clinical follow up information.

Design: 1,835 cases with the following criteria were identified using a retrospective computerized search of clinical test results from studies performed from 2003 – 2006: 1) valid results of both UroVysion and cytology from the concurrent urine samples, and 2) histological and/or cystoscopic follow up within 4 months of the original tests, or 3) at least 3 years of clinical follow up information. The sensitivity, specificity, positive and negative predictive values of UroVysion and urine cytology in detecting UCC were obtained by comparison with the "gold standard" that is derived from the combination of histological, cystoscopic and clinical follow up information. A clinical positive was defined as UCC confirmed by the concurrent or follow up histology and cystoscopy, or clinically known UCC. A clinical negative was defined as negative histological and cystoscopic findings, or without evidence of UCC during at least a 3 year clinical follow up period.

Results: The correlation of UroVysion and cytology results with clinical findings is shown in Table 1. The overall sensitivity, specificity, positive and negative predictive values for UroVysion in detecting UCC were 61.2%, 89.5%, 42.6% and 92.4%, respectively, for cytology 43.6%, 96.5%, 63.8% and 92.0%, respectively; and for combination of the two tests 73.4%, 86.7%, 50.4% and 94.6%, respectively. The sensitivity of both tests was better for detection of high grade UCC (UroVysion 75.0% and cytology 55.7%) and it was much lower for low grade UCC (UroVysion 46.8% and cytology 20.7%).

The Correlation of UroVysion and cytology with Clinical Information

	No. of Cases	UroVysion +	UroVysion -	Cytology +	Cytology Atypical	Cytology -
Clinical Positive	291	178	113	83	96	112
Clinical Negative	1544	162	1382	47	202	1295

Conclusions: UroVysion is more sensitive than cytology in detecting UCC in all categories examined, especially in low grade UCC. Combination of UroVysion and cytology further improved the sensitivity without significant affecting the specificity.

458 Experience with Testicular Touch Preparation Cytology in the Evaluation of Male Infertility.

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Background: Male infertility is traditionally evaluated by tissue core biopsies of the testes. Touch preparations of these biopsies and fine needle aspiration of the testis have also been used in the past. The efficacy of testicular touch preparation (TP) cytology has not been widely reported. The aim of this study was to report our experience with using testicular biopsy TPs in the evaluation of male infertility.

Design: A retrospective search of our pathology computer database was conducted (2004 to 2010) for cases of testes biopsies with concurrent TP submitted to our department. These cases were further evaluated for clinical information (patient age, indication for biopsy), specimen adequacy, and cytological-histological correlation. In all cases, 6 core biopsies were performed from upper, middle and lower regions of bilateral testes with corresponding TP (6 TP slides/case with Pap stain). Slides from selected cases were reviewed.

Results: A total of 35 cases were identified from men with a mean age of 34 years (range 23 to 50 years). Clinical indication for biopsy included atrophic testes, azoospermia, Klinefelter syndrome, scrotal varices, testicular failure, and rule out carcinoma in situ. Histopathological diagnoses included 4 (11%) cases with complete spermatogenesis, 4 (11%) with hypospermatogenesis, 10 (29%) maturation arrest, and 17 (49%) with absent spermatogenesis, of which 5 (29%) were due to Sertoli cell-only syndrome. No intratubular germ cell neoplasia was diagnosed, confirmed using immunohistochemical studies in 4 cases with germ cell atypia. TP slides were satisfactory for evaluation in 31 (89%) cases, and less than optimal in 4 due to low cellularity, blood or air drying artifact. Cytopathology showed concordance with biopsy in all (100%) cases.

Conclusions: TP of the testis is a helpful adjunct to biopsy because of its ability to evaluate all stages of spermatogenesis. These data demonstrate that TP cytopathology of the testes in our experience has excellent correlation with both normal testicular biopsies and those showing pathologic spermatogenesis. Albeit uncommon, cytopathologists may be required to identify and evaluate spermatogenic elements in cytology specimens being submitted from men with infertility.

459 Diagnosis of Metastasis to the Pancreas by Fine Needle Aspiration Biopsy.

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Background: Correctly distinguishing primary tumors of the pancreas from metastases to the pancreas is important, given the sometimes widely different treatment regimens. However, metastases to the pancreas are relatively uncommon, and little information is available in the literature. Here we report the twenty-year experience of cytological diagnosis of pancreatic metastases.

Design: All cases diagnosed by endoscopic ultrasound-guided (EUS) fine needle aspiration (FNA), computed tomography (CT)-guided percutaneous FNA, and ultrasound-guided percutaneous FNA between January 1, 1990 and March 30, 2010 with the diagnosis of metastases to the pancreas were identified by searching the clinical database. Imaging and pathologic features were collated.

Results: We identified 57 cases of metastasis to the pancreas. Thirty-seven specimens were obtained by EUS-FNA, thirteen by CT-guided percutaneous biopsy, and seven by ultrasound-guided percutaneous biopsy. In order of decreasing frequency, the primary site was identified as hematopoietic (21), kidney (11), lung (7), skin (6), ovary (3), breast (2), soft tissue (2), esophagus (2), colon (1), nasopharynx (1), or prostate (1). The five most common tumors to metastasize to the pancreas were, in descending order, non-Hodgkin B-cell lymphoma, renal cell carcinoma, melanoma, neuroendocrine carcinoma of the lung, and adenocarcinoma of the ovary. In 46% of cases (26 of 57), the pre-biopsy imaging findings favored metastasis. In 19% of cases (11 of 57), metastasis was not favored by the imaging findings, and in the remaining 35% cases (20 of 57), the suspected primary site was either equivocal or was unspecified. In 81% of cases (46 of 57), the pancreatic tumor was able to be definitively classified as metastatic after cytopathologic evaluation, while the remaining 19% (11 of 57), mostly comprised of poorly-differentiated carcinomas of uncertain histogenesis or of scant specimens unable to be definitively classified, were considered to be probable metastases. Of these eleven cases, histological follow up was unavailable for nine, while two had histological confirmation as metastases.

Conclusions: Metastases to the pancreas are infrequent and are not always classifiable as such by imaging. Cytopathologic evaluation is useful in distinguishing primary from metastatic tumors of the pancreas in the majority of cases.

460 Diagnosis of Central Nervous System (CNS) Involvement of T Cell Lymphoproliferative Diseases from Cerebrospinal Fluid (CSF) with Combined Cytology, Flow Cytometry and Lymphoid Antigen-Cell Receptor Antigen Rearrangement Studies.

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Background: Leptomeningeal involvement of the CNS by a T cell lymphoproliferative disorder tends to be allied with a worse prognosis. The diagnosis of intrathecal and CNS involvement is often defined by the clinical presentation and/or the presence of neoplastic cells in the CSF cytology specimen. However, cytological recognition of neoplastic lymphoreticular cells in CSF is often difficult and the sensitivity can be rather low. Ancillary studies of CSF such as flow cytometry and PCR evaluation of lymphoid cell antigen-receptor gene rearrangement are often helpful. The aim of this study was to investigate whether cytofluorographic and molecular studies increase the sensitivity and specificity of diagnosis of lymphoproliferative disorders involving the CNS.

Design: Cases with positive T-cell antigen-receptor rearrangement from CSF over the last 7 years were reviewed. The clinical histology, cytology results, flow cytometry and PCR gene rearrangement studies were reviewed and compared.

Results: Twenty-five (25) cases with 38 samples of CSF showed T-cell receptor antigen rearrangement. Among them, 20 (75%) patients (32 out of 38 samples, 84.2%) presented with clinical signs such as vomiting, lethargy, with morphology of a lymphoproliferative disorder either T cell (14 cases) or B cell (6 cases). The cytology specimen showed blast or atypical lymphocytic process in 6 out of 25 cases (24%), 15 out of 38 samples (39.5%). Flow cytometry revealed abnormality in 9 out of 25 cases (36%), 18 out of 38 samples (47.4%). 5 cases patients do not have any lymphoproliferative disorders. (1 patient has squamous cell carcinoma, 2 HIV infection, 1 sarcoidosis, 1 optic neuritis).

Conclusions: Using ancillary studies such flow cytometry and PCR for T cell receptor antigen rearrangement increases the sensitivity of diagnosis of CNS involvement of T lymphoproliferative disorders over standard cytology. However, when analyzing the results, pathologists have to be aware of the potential for false positivity of the molecular study. Thus, clinicians and pathologists have to put together the entire picture including the clinical symptoms, cytology, flow cytometry and molecular studies when making a diagnosis of a T lymphoproliferative disorder involving the CNS.

461 Value of PAX8 and WT1 Immunostains in the Detection of Metastatic Ovarian Carcinoma in Effusion Specimens.

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Background: Diagnosing metastatic ovarian carcinoma (OC) in effusion samples based solely on cytomorphologic features can be challenging, because the tumor cells can resemble reactive mesothelial cells. WT1 immunostaining is often used to aid diagnosis in this setting. Recently, PAX8 immunostaining was found to be positive in most primary OC; however, its role in detecting metastatic OC in effusion samples has not been well defined. The aim of this study was to compare the diagnostic value of PAX8 immunostaining with that of WT1 immunostaining.

Design: Between January 2002 and January 2010, we identified 68 peritoneal or pleural effusion samples with metastatic OC. All patients had history of primary OC confirmed by surgical excision but no history of other malignancy. Cell block sections (n=45) or direct smears (n=23) were used for immunostains for PAX8 and WT1. Nuclear staining in >5% tumor cells was considered positive.

Results: Histologic diagnoses of the 68 primary OC included serous carcinoma (SC) in 52 cases (46 high-grade and 6 low-grade), clear cell carcinoma in 5, borderline mucinous neoplasm in 1, malignant mixed Mullerian tumor (MMMT) in 1, and carcinoma with mixed components of SC, endometroid, clear cell, transitional, and undifferentiated carcinoma in 9. PAX8 staining was positive in 57 (84%) cases, and WT1 staining was positive in 58 (85%) cases (Table1); 50 (74%) cases were positive for both markers. Combination of both markers yielded a detection rate of 94% (64/68). Detection rates on the cell block and on smear were 91% and 78% for PAX8, and 82% and 83% for WT1. Notably, in 21 (31%) cases, WT1 was also positive in the background mesothelial cells.

Table 1. Detection Rate for Metastatic Ovarian Cancer in Effusion Samples by PAX8 and WT1 Staining

		Number (%) of Positive Effusion Sample	
Surgical diagnosis of primary ovarian tumor	Total Cases	PAX8	WTI
High-grade SC	46	42 (91%)	42 (91%)
Low-grade SC	6	3 (50%)	6 (100%)
Clear cell carcinoma	5	5 (100%)	1 (20%)
Borderline mucinous neoplasm	1	0 (0%)	0 (0%)
MMMT	1	1 (100%)	1 (100%)
Carcinoma, mixed	9	6 (67%)	8 (89%)
Total	68	57 (84%)	58 (85%)

Conclusions: PAX8 and WT1 had similar overall detection rates for metastatic OC in effusion samples. PAX8 was more readily than WT1 to detect clear cell carcinoma but was less effective at detecting low-grade SC. Combining PAX8 and WT1 substantially increased the diagnostic accuracy. Both cell block sections and smears can be used for PAX8 and WT1 staining.

462 Urine Cytomorphology of Micropapillary Urothelial Carcinoma.

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Background: Micropapillary urothelial carcinoma (MPUC) is a rare variant of UC with an aggressive clinical course in terms of higher T stage, higher incidence of lymphovascular invasion, and higher incidence of lymph node and/or distant metastasis (our data not shown here). However, cytologic features of MPUC in urine cytology have not been well described. The aims of this study were to describe cytologic features of MPUC and compare them with those of high grade UC (HGUC).

Design: 21 urinary specimens (11 voided and 10 washings) from 14 patients with diagnosis of MPUC on follow-up surgical specimens, and 28 specimens (14 voided and 14 washings) from 28 patients with HGUC were retrieved. The cytologic features, single cell patter, papillary architecture, flat sheets/nests, 3 dimensional clusters, micropapillary (inside-out, acinar-like), nuclear grade, cytoplasm quantity, cytoplasmic vacuoles, and necrosis, were revaluated. Clinical follow-up was also reviewed.

Results:

Table 1. Cytologic features of MP-UC and HG-UC.

	Single Cells	Papillary architecture	Flat Sheets	3-Dimensional Clusters	Micro-papillary	Nuclear Grade	Cytoplasmic vacuoles
MP-UC	100%	47.6%	38.1%	85.7%	81.0%	2.7 ± 0.6	47.1%
HG-UC	100%	35.7%	35.7%	85.7%	14.3%	2.3 ± 0.7	14.3%
P Value	1.000	0.099	0.181	0.186	< 0.001	0.026	< 0.01

Chi Square test.

Table 2. Re-evaluation of urine specimens.

	MP-UC			HG-UC		
	Original	Re-evaluation	Possible MP	Original	Re-evaluation	Possible MP
UC	17/21 (81%)	20/21 (95%)	17/21 (81%)	13/28 (46%)	22/28 (79%)	4/28 (14%)
Suspicious for UC	1/21 (5%)		P < 0.001	2/28 (7%)	3/28 (11%)	
Atypical UC	1/21 (5%)	1/21 (5%)		6/28 (21%)	3/28 (11%)	
Negative	2/21 (10%)			7/28 (25%)		

Chi Square test.

8/14 (57%) MPUC and 1/28 (4%) HGUC showed metastasis to lymph node and/or distant organs (P < 0.001).

Conclusions: 1. On urine cytology, micropapillary architecture and cytoplasmic vacuoles are the two most useful features for the diagnosis of MPUC with sensitivity of 81%, specificity of 82%, positive predictive value of 77% and negative predictive value of 85%.

2. Nuclear grade of MPUC is slightly higher than that of HGUC.

3. MPUC can present with single cells, papillary architecture, flat sheets/nests, 3 dimensional clusters, and necrosis in urine cytology as seen in HGUC.

4. Patients with MPUC show higher incidence of lymph node and distal metastasis than HGUC (57% vs. 4%).

5. Careful evaluation of urine cytology for possible MPUC is very helpful to guide clinicians in choosing deep biopsy for possible MPUC cases, and predicting metastasis and prognosis.

Dermatopathology

463 Lymphatic Invasion in Melanocytic Tumors of Uncertain Malignant Potential May Predict Worse Outcomes.

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Background: Melanocytic tumors of uncertain malignant potential (MELTUMPs) are a subset of difficult melanocytic lesions that evade consensus from novice and expert alike. These lesions are bulky dermal melanocytic tumors that include atypical forms of spitzoid, cellular blue, and deep penetrating nevi, with features of both benign nevi and malignant melanoma. Previous studies of these lesions have shown that patients generally have favorable outcomes, with features such as mitotic activity, mitotic activity near the base, and inflammatory infiltration of the tumor as characteristics that may predict a more aggressive course. Lymphatic invasion has been found to a potential predictor of regionally metastatic disease in malignant melanoma. We attempt to test whether lymphatic invasion in MELTUMPs may be indicative of a worse prognosis.

Design: Fifty-five melanocytic lesions diagnosed as MELTUMPs at our institution and elsewhere were procured. Dual immunohistochemical staining for D240 and S100 is performed on the unstained tissue sections. Clinical follow up has been obtained for some of the patients.

Results: Of the thirty four cases of MELTUMPs currently stained, lymphatic invasion was found in 6 of them (6/34, 17.6%). Clinical history is currently obtained for 12 patients, with the current average clinical follow up being 10.9 years. Of these 12, four patients have clinical evidence of having a malignant course, and two of these patients have lymphatic invasion by dual immunohistochemistry (2/4, 50%).

Conclusions: Our preliminary data thus far shows that lymphatic invasion found by our method is quite promising as a potential tool to predict which MELTUMPs are more likely to develop a malignant course. Additional staining and clinical information gathering is currently ongoing to complete the study.

464 Deep Dermal Fungal Infections in Patients That Are Immunosuppressed.

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Background: Most clinicians use acute and granulomatous inflammation as a threshold for suspicion of an infectious organism. In immunosuppressed patients that are unable to mount an adequate immune response it has been postulated that the threshold for suspicion should be much lower. Over the years few journal articles have been published on characterizing fungal infections of the skin and the presentation in immunosuppressed patients. Most of these publications are case reports on opportunistic fungi such as *Fusarium*, melanized fungi, and *Cryptococcus*. Our goal is to look at a population of immunosuppressed patients and characterize the leading pathogens and compare the findings to those of immunocompetent patients in the literature.

Design: All the patients were selected from the Mayo database over the past 10 years. The inclusion criteria were that all patients will have had to have had a fungal infection of the skin that has been biopsied and also had a positive fungal culture. These patients will also have any kind of history that would lead to immunosuppression, whether it be by transplantation, treatment for an autoimmune condition, lymphoma, congenital/acquired immune condition, ect. All of the cases had a GMS (Grocott's Methanamine Silver) and/or PAS stain performed.

Results: Our initial results show that among 7 patients, *Alternaria* sp. was the most common organism affecting 3 patients. The remaining 4 patients were infected by *Exophilia jeanselmei*, *Scedosporium apoispermum*, *Fusarium* sp., and *Aspergillus* sp. A variety of host responses were observed ranging from a suppurative granulomatous response to a paucicellular immune response and mycetoma formation. The clinical presentations were predominantly nontender nodules that were biopsied to rule out carcinoma.

Conclusions: One of these patients had a large deep dermal nodular area with paucicellular lymphocytic infiltrate and necrotic debris that could be mistaken as infarct. Due to the patient's history, a GMS stain was ordered which revealed the entire area to be mycetoma which was initially very difficult to descern on H&E. This scenario could represent another diagnostic pitfall should there be low clinical suspicion.

465 Tyrosinase-Related Protein2 (trp2) Is a Melanocyte Differentiation Antigen (MDA) Useful for Surgical Pathology.

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Background: MDAs are expressed in cells and tumors of melanocytic lineage. MDAs such as gp100, Melan-A/MART1, and tyrosinase and their corresponding antibodies HMB45, A103, and T311 serve as important diagnostic tools in surgical pathology and are also employed as vaccine targets for the immunotherapy o malignant Melanoma (mM). However, little is known about trp2, another MDA, functionally a Dopachrome Tautomerase (DCT). In the present study, we determined the specificity of a novel anti-trp2 mAb clone C9 and analyzed the expression of trp2 in panels of normal and tumor tissue.

Design: MAb C9 to trp2 was obtained commercially. C9 was tested for specificity by Western Botting (WB) and ELSIA with rt-PCR tested cell lines and mM specimens. Its suitability for IHC was tested in in cell line pellets as well as in panels of normal and tumor tissues pre-typed by rt-PCR.

Results: In WB and ELISA, mAb C9 was reactive solely with trp2 mRNA-positive cell lines and tissues as well as the trp2 protein respectively but not with trp2-negative cell lines and not with unrelated proteins. In IHC, mAb C9 worked well in frozen and FFPE tissue employing antigen retrieval. In skin, typical staining of the melanocytes was present. Immunostaining of mAb C9 was completely inhibted by blocking with trp2 protein and not with other proteins. IHC of mM cell lines corresponded to the trp2 mRNA expression. 16/19 (84%) primary mMs and 19/33 (64%) metastatic mMs were C9 positive, both showing a mostly homogenous staining pattern. Several HMB45-negative cases, were trp2-positive. 10/10 mucosal mM were also trp2 positive. 6/6 desmoplastic mMs were negative. C9 reactivity was only focally present in only 2/9 angiomyolipomas. Besides melanocytes, no C9 reactivity was seen in normal tissues. Panels of non-melanocyic tumors such as carcinomas of colon, breast, lung, ovary, kidney and several sarcoma types were all C9-negative.

Conclusions: MDAs are important diagnostic tools as well as potential vaccine targets for the immunotherapy of mM. Here we show that mAb C9 is a novel specific marker for the detection of trp2. Expression of trp2 in mM parallels other MDAs such as Melan-A and tyrosinase being present in a high percentage of primary and metastatic mMs. Trp2 is a useful diagnostic marker and a potential vaccine target for melanoma.

466 Definition and Categorization of Combined Melanocytic Nevi.

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Background: "Combined nevus" is a term used to describe tumors composed of two or more distinct populations of melanocytes. While this term has historically been used to describe the combination of blue nevi with common nevi, it has more recently been applied to other combinations of benign melanocytic proliferations. We sought to