329 Lymphoplasmacytic Response to Atheroma Mimicking Vasculitis

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Background: Coronary artery atheroma accounts for the vast majority of sudden cardiac deaths whilst coronary artery vasculitis accounts for a rare number. We report a series of atheromatous coronary arteries with associated florid lymphoplasmacytic inflammation mimicking vasculitis.

Design: Three cases with mass like lesions surrounding atheromatous coronary arteries were referred from a single centre to the National Heart and Lung Institute at the Royal Brompton Hospital for expert pathology review. The cases were from males with mean age 74 years (range 55 – 91). In all cases coronial autopsies were carried out for sudden deaths in the community. Past medical histories of note were hypertension (N=2) and ischaemic heart disease (N=1), with one patient having a past history of aortic aneurysm repair.

Results: At autopsy, firm, white and whorled masses were described surrounding atheromatous coronary arteries ranging in size from 9-25mm in diameter. Each coronary artery had intimal atheroma ranging from moderate to severe. A thrombus was identified in one case. No gross infiltration of the myocardium was seen. No vascular abnormalities or lesions were identified elsewhere. Histological sections showed a mixed inflammatory infiltrate extending from the media into the adventitia, composed predominantly of plasma cells and lymphocytes with rare neutrophils and eosinophils. No giant cells or epithelioid cells were noted. No necrosis was present. There was focal infiltration of the myocardium by lymphoid aggregates. There was accompanying dense fibrosis accounting for 50% of the mass size. The presence of intimal circumferential atheroma was confirmed in all cases. Myocardial fibrosis was identified in one case. No inflammatory infiltrate was present in the myocardium. Slides from all three cases were stained with IgG and IgG4 by immunohistochemistry. The stained cells were counted in three high power fields from areas with the highest density of plasma cells using a 40x objective and 10x eyepiece of an Leica microscope. The proportion of IgG4 expressing plasma cells was greater than 50% of IgG-expressing cells in two of the three cases.

Conclusions: Atheroma may be associated with mild chronic inflammation present in the intima or associated with plaques. IgG4 related disease has been described in various organ sites, we report a series of IgG4 related coronary artery vascular lesions in association with atheroma.

330 Collagen Alterations in the Dissecting Aortic Aneurysm (DAA) Caused by N-(2-Aminoethyl) Ethanolamine (AEEA) in Rat

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Background: The industrial chemical N-(2-aminoethyl) ethanolamine (AEEA) induces dissecting aortic aneurysm (DAA) in newborn rats following *in utero* exposure. To extend our knowledge about the severe deleterious effects of AEEA on normal vascular development, we aim to study the extracellular matrix changes during the formation of developmental DAA.

Design: We treated pregnant Sprague-Dawley rat dams with AEEA by gavage on gestation day (GD) 14-20, a period of rapid aortic development (Doses: 10, 50, 100 and 150 mg/kg/day; controls received saline only). Aortas from fetuses on GD 20 and from newborn pups (day 1) were examined by histopathology. Total collagen in aorta of fetuses from dam treated with AEEA (150 mg/kg/day) was examined by multiphoton fluorescence (MPF) and second harmonic generation (SHG) microscopy. Collagen types 1 & 3 distribution and quantization in aorta were further studied by immunohistochemistry and native western blot respectively.

Results: GD 20 fetuses showed no lesions. Extensive DAA was found in 100% of newborn pups (post natal day 1) in the two highest dose groups (100 and 150 mg/kg/day). Mediastinal hemorrhage, dissection in pulmonary artery and carotid artery were also found. A lesion grading system was devised; a dose-reponse was demonstrated for severity of lesion grade with AEEA dosage. Multiphoton fluorescence (MPF) and second harmonic generation (SHG) microscopy showed total collagen in aorta of GD 20 fetuses from treated dams was decreased. GD 20 fetuses from treated dams showed a decreased content of medial and adventitial collagen types 1 & 3 in aorta by immunohistochemistry; this decrease was confirmed by native western blot in pooled aortas. Collagen types 1 & 3 in AEEA treated group significantly decreased 34% and 30% respectively. There was no significant change of collagen types 1 & 3 of skin in AEEA treated group compared to the control, indictating that the collagen pathophysiology induced by AEEA exposure is specific for aorta and not a generalized phenomenon.

Conclusions: The pathologic mechanism of AEEA induced developmental DAA may be related to decreased collagen types 1 & 3 weakening the vascular wall. DAA induced by AEEA is a reproducible model that is useful to study the underlying mechanisms of DAA development *in vivo.*

331 Incidental Ascending Aortitis: The Histologic and Clinical Spectrum

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Background: The incidence of aortitis in series of aortic aneurysm repair is between 5 and 8%. In most cases, the diagnosis is made initially by histopathologic evaluation of the specimen. There are few single-institution clinicopathologic series of aortitis.

Design: We prospectively studied histologic features of aortitis in series of repaired aortic aneurysms over a 6-year period with clinical correlation and follow-up.

Results: Of 254 aortic resections, there were 17 cases of incidental aortitis (6.7%); 9 women (74 ± 13 years) and 8 men (62 ± 15 years). 4 patients had prior autoimmune disease (rheumatoid arthritis, giant cell arteritis, ankylosing spondylitis, and IgA nephropathy); 1 was diagnosed subsequently with Takayasu disease. Additional 3 patients had positive rheumatoid factor and ANA, history of Lyme disease, and fibromyalgia. Grossly, all cases of aortitis showed a distinct wrinkled intima. The surgeon noted abnormal thickened aortic wall intraoperatively in 8 cases. Histologically, there were two types of aortitis: necrotizing aortitis and periaortitis. Necrotizing aortitis demonstrated three phases: acute, healing, and healed. In the acute phase (n=3), there were linear zones of necrosis > 3 mm with peripheral predominately adventitial macrophage giant cells; 2 cases also showed pockets of neutrophils. In the healing phase (n=5), there was both zonal medial necrosis < 3 mm with surrounding granulomatous reaction, and areas of healing with smooth muscle cell proliferation, often with proteoglycans mimicking cystic medial degeneration. In the healed phase (n=7), only the latter changes were noted. All cases showed brisk adventitial chronic inflammation and scattered inflammatory infiltrates around medial vessels. The two cases of periaortitis had numerous (>25 per hpf) IgG4 plasma cells and adventitial fibrosis. Intimal and adventitial thickening was mild in necrotizing aortitis (mean 0.6 and 0.9 mm, respectively) and greater in IgG4 disease (mean 4.2 and 1.3 mm, respectively). 2 patients with necrotizing aortitis progressed with new descending aortic aneurysms. One of these patients was treated with immunosuppressive treatment and one with anti-inflammatory drugs; they were the only patients given systemic treatment.

Conclusions: Aortitis is the cause of about 7% of surgically repaired ascending aortic aneurysms. Most cases show necrotizing medial inflammation that heals with a characteristic histologic appearance. IgG4 disease is a less common cause of aortitis that involves primarily the adventitia.

332 Hypersensitivity Myocarditis before Heart Transplantation Is Associated with Increased Acute Cellular Rejection after Transplantation

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Background: Hypersensitivity myocarditis (HSM) is associated with the use of multiple drugs, and has been occasionally observed in the patients with end-stage heart failure. However, whether or not HSM at the time of heart transplantation (HTx) affects long-term prognosis including acute cellular rejection (ACR) and antibody-mediated rejection (AMR) after HTx remains unclear. This study addresses the occurrence, clinical and pathological characteristics, and prognosis of patients with HSM at HTx.

Design: 766 consecutive patients who underwent de-novo HTx at Columbia University Medical Center between 2000 and 2010 were retrospectively reviewed. Clinical characteristics and pathological findings of patients with pre-HTx HSM diagnosed by histological evaluation of the explanted heart were analyzed. Prognosis after HTx was compared between the patients with or without HSM.

Results: HSM was observed in 21 patients (2.7%). The rate of pre-HTx HSM in our hospital has been decreasing during the study period in inverse relation to the rate of left ventricular assist device implantation before transplant. 19 patients (90%) had peripheral blood eosinophilia at the time of HTx, but in no case was HSM clinically diagnosed. Dobutamine, a common cause of HSM, was administrated in 12 patients (57%). All patients had varying degrees of mixed inflammatory infiltrates with eosinophils, lymphocyte, macrophages, and plasma cells, but none of the patients showed myocardial necrosis. The number of episodes of biopsy diagnosed ACR (ISHLT grade ≥2R) was 11 (3.9%) in HSM patients and 197 (2.2%) in patients without HSM (p=0.06) during the first year post-HTx, and 11 (3.8%) in HSM patients and 78 (1.5%) in patients without HSM (p=0.006) after second year post-HTx. Regarding AMR, there was no statistically significant difference between the groups throughout the study period. Post-transplant survival did not differ in patients with or without pre-transplant HSM.

Conclusions: HSM at the time of HTx is associated with an increased frequency of late ACR after HTx. Post-HTx survival is not influenced by pre-transplant HSM.

Cytopathology

333 Intra-Abdominal Neuroendocrine Tumor Diagnosed by Endoscopic Ultrasound-Guided Fine Needle Aspiration (EUS-FNA): A Retrospective Study from an Academic Tertiary Center

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Background: Neoplastic lesions of the pancreas, liver, retroperitoneal lymph nodes can be better visualized and appropriately sampled with fine-needle aspiration biopsy by endoscopic ultrasound (EUS-FNA). Diagnosis of pancreatic endocrine tumor can be rendered, in most cases, without difficulty based on cytomorphologic and immunophenotypic features. This study reviews the usefulness and accuracy of EUS-FNA in the diagnosis of neuroendocrine tumor (NET) in pancreas, liver, and lymph nodes.

Design: This is a retrospective study. Intra-abdominal EUS-FNA specimens (2003-2012) with a NET were retrieved. Metastatic small cell carcinoma was excluded. Immunohistochemistry study (small panel: CD57, CD56, synaptophysin, chromogranin A and B) was performed when cell block was available. Cases were classified as consistent with NET, possible/suggestive of NET, few cells with neuroendocrine differentiation. Clinicopathologic data were collected. On site cytopatholgic evaluation was done in all cases.

Results: We retrieved 148 patient specimens. Overall accuracy of EUS-FNA compared to definitive histopathology of surgical specimen was 85% (81/95). In 14 cases, there was little discrepancy. Final diagnosis was: Paragangliomas (3 cases); 1 intra pancreatic and 2 retroperitoneal; composite carcinoma with ductal, acinar, endocrine components (5 cases); Schwannoma (1 case); Metastasis from acinar cell carcinoma (1 case); Solid

pseudopapillary tumor (3 cases); And 1 case with chronic pancreatitis. The definitive histopathology on surgical specimen was not available for 53 cases (surgery not indicated or patients scheduled for surgery).
Conclusions: Diagnosis of neuroendocrine tumor on EUS-FNA of intra-abdominal masses was rendered when cytomorphologic features were clear. Uncommon cytomorphologic features may impose a diagnostic challenge expending the differential diagnosis to include: acinar cell carcinoma, solid pseudopapillary tumor, composite carcinoma and metastatic neuroendocrine carcinoma. Distinction may have clinical implications. Correlation of cytomorphologic features and ancillary studies (a larger mmunohistochemistry panel when sufficient material is available) with the clinical and radiological information are essential to establish an accurate diagnosis.

334 T-Cell Lymphoma Is a Rare Entity in Body Fluid Cytology: Findings from a Large Case Series

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Background: Malignant lymphomas may cause effusion fluids during the course of the disease. Moreover, lymphomas may initially be diagnosed in body fluid samples. Cytomorphology in conjunction with flow cytometric immunophenotyping is essential in evaluation of these specimens. Both B and T cell lymphomas (TCL) are reported in association with effusions. The incidence of body fluid specimens involved by T cell lymphomas is unclear. Only a handful of case reports and case series describing various T cell lymphomas in the body fluids are identified in the literature, without an up-to-date immunophenotypic and genotypic characterization of the (TCL) subtype.
Design: We are reporting a large series of body fluids to identify and characterize TCL using the current WHO Classification, 2008. We identified 9,969 body fluid specimens in the database of two major academic centers over a period of 2005 -2010. The cytologic, flow cytometric, immunohistochemical, cytogenetic and molecular genetic studies of these cases were reviewed.
Results: 53 of 9,969 body fluid specimens were involved by lymphoma; 41 B cell lymphoma (0.4%) and 12 T cell lymphoma (0.1%). The 12 samples with TCL belonged to 8 patients and included; pleural effusion (n = 7), ascitic fluid (n = 2), bronchoalveolar lavage fluid (n = 2) and pericardial effusion (n = 1). The patients were predominantly male (6 males, 2 females), age range 9-71 yrs. Flow cytometric immunophenotyping was performed in 6 out of 12 samples (50%). In 3/8 patients, primary diagnosis of lymphoma was established in the body fluid specimen. The rest of the patients (5) were diagnosed with TCL previously from biopsies of: gastric mass (n = 1), lymph node (n = 1), abdominal mass (n = 1), lung (n = 1) and bone marrow (n = 1). The diagnoses were Adult T-cell leukemia/lymphoma (3), ALK+ Anaplastic Large Cell lymphoma (2), Angioimmunoblastic T cell lymphoma (1), Peripheral T cell lymphoma, Not Otherwise specified (1) and T lymphoblastic lymphoma/leukemia (1). Cytogenetic and molecular genetic studies were performed in 3 patients. Serologic studies were positive for HTLV-1 antibodies in three patients.
Conclusions: In our series, body fluids involved by TCL represented only 0.1% of the specimens. Most common type of TCL was HTLV-1 positive ATLL followed by ALK+ ALCL. In spite of rarity and variable cytomorphologic presentation of the TCL, primary as well as the secondary diagnosis can be made with confidence using cytomorphologic and flow cytometric evaluation. Cytogenetic, molecular genetics and serologic studies are a critical part of the evaluation of body fluids for TCL.

335 Carbonic Anhydrase IX (CAIX) Expression Does Not Discriminate between Benign and Malignant Effusions

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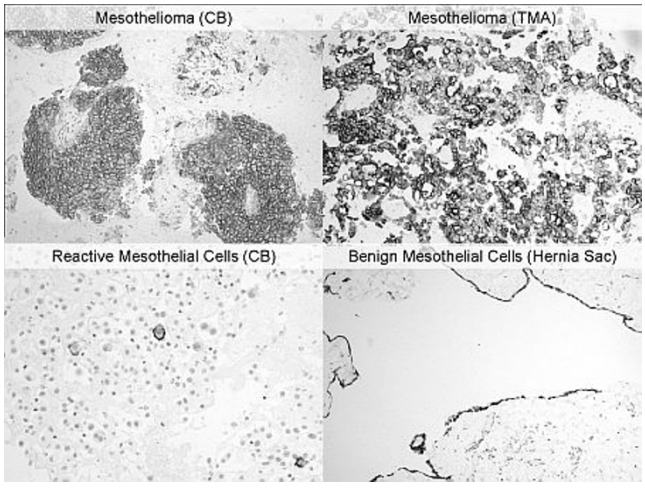
Background: Carbonic anhydrase IX (CAIX), a commonly used diagnostic immunohistochemical stain for clear cell renal cell carcinoma (RCC), has recently been described as a marker for distinguishing benign from malignant effusions. In these studies, benign pleural effusions were noted to be negative for CAIX expression, in contrast to atypical and malignant effusions. In the current study, we evaluated expression of CAIX by immunohistochemistry (IHC) on archival cell blocks (CB) from pleural and peritoneal fluids, benign mesothelium from hernia sacs and mesothelioma tissue microarrays (TMA).
Design: Pleural and peritoneal fluids with the following diagnosis: reactive (n=9), carcinoma (n=25) and suspicious for mesothelioma (n=4) were stained for CAIX, calretinin, BerEP4 and MOC31. The "carcinoma" category included metastasis from various primary sites including lung, ovary, breast, prostate and kidney. A mesothelioma TMA comprising of epithelioid (n=32), well differentiated papillary (n=1) and sarcomatoid (n=7) variants and 3 cases of benign mesothelium (hernia sacs) were also immunostained for CAIX. All immunostains were primarily evaluated for presence or absence of staining. For the fluids, calretinin and CAIX immunostaining pattern were compared.
Results: In the fluid specimens, CAIX reactivity was noted in 5 of 9 reactive and 11/29 malignant effusions (p=not significant). In carcinomatous effusions, CAIX expression was restricted to benign mesothelial cells while carcinoma cells were negative. All fluids categorized as "suspicious for mesothelioma" showed CAIX reactivity. Of all fluids, 86% showed similar numbers of CAIX and calretinin expressing cells. In tissue specimens, CAIX immunoreactivity was noted in all benign mesothelial tissues (3/3) and majority of mesotheliomas (34/40). Of the mesotheliomas, epithelioid variants most consistently expressed CAIX (29/32), while 4 of 7 sarcomatoid variants were also positive.



Conclusions: CAIX can serve as an ancillary marker for identifying cells of mesothelial lineage. There is no difference in CAIX expression between benign and malignant mesothelial cells. In carcinomatous effusions, only the mesothelial cells immunoreact for CAIX. CAIX cannot be used as a definitive marker for metastatic carcinoma.

336 Ultrasound Guided Fine Needle Aspiration (US-FNA) of Thyroid Nodules by a Cytopathologist at the University of Toledo Medical Center. Lessons from a Northwest Ohio Hospital

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Background: The current state of the art method to sample thyroid nodules is by ultrasound guided fine needle aspiration (US-FNA). Our study evaluated the accuracy, safety, and results when this diagnostic modality is managed by an interventional cytopathologist.
Design: Thyroid US-FNAs performed by a cytopathologist between October 2010 and September 2012 were retrospectively reviewed. Clinical data, ultrasound features, duration of the procedure, number of passes, complications, adequacy, diagnosis, histologic correlation, and turn around times were analyzed. The aspirated material was obtained using a combination of Swedish and American methods with a 25 G x 1½ needle. Smears were stained for immediate evaluation with Diff-Quik. Additional smears were fixed in alcohol for Papanicolaou stain. Preliminary interpretations were incorporated in our clinical portal computer system within 2 hours after the procedure. The reports included a final diagnosis, Bethesda guidelines recommendations, clinical data, laboratory test results, ultrasound findings, procedure notes, and a microscopic description.
Results: A total of 164 thyroid US-FNAs from 112 patients were evaluated. The size of the nodules ranged from 0.5 to 7.5 cm with an average size of 2 cm. The average duration of the procedure was 45 minutes. Nodules were sampled an average of 3 times with the number of passes ranging from 1 to 5. The minor complication of a small hematoma occurred in 1 case (0.6%). All samples were adequate for evaluation. Of the 164 cases, 134 (82%) were benign, 25 (15%) were atypical/borderline, and 5 (3%) were malignant. There were 8 (6%) cytologically benign cases with tissue correlation, all which were histologically confirmed as such. Of the 25 atypical/borderline cases, 13 (52%) had tissue correlation. This included 10 cases of nodular hyperplasia with superimposed Hürthle cell metaplasia, 2 cases with radiation changes, and 1 case of metastatic leiomyosarcoma. Of the 5 malignant cases, 4 (80%) had tissue correlation. In every case the cytologic diagnosis was confirmed, including 1 case of metastatic leiomyosarcoma and 3 cases of papillary thyroid carcinoma. A final diagnosis was reported in 94% of the cases within 2 business days with the majority (69%) of those cases being reported in one business day or less.
Conclusions: Using a personalized multidisciplinary approach US-FNA performed by an interventional cytopahtologist can be a particularly safe, accurate, fast, and effective diagnostic modality.

337 Ongoing Physician Performance Evaluation (OPPE) for Clinicians Performing Endoscopic Ultrasound (EUS) and Endobronchial Ultrasound (EBUS) Guided FNAs

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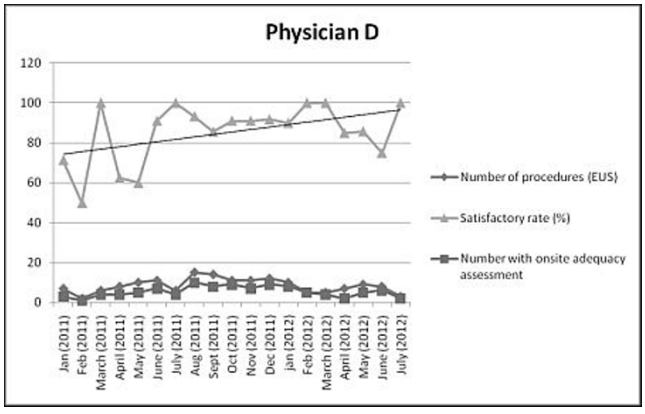
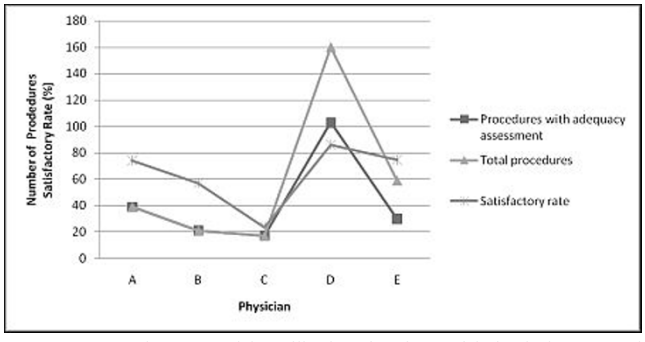
Background: OPPE is mandated by the Joint Commission. Two requirements for OPPE include (1) clearly defined performance measures (PM) and (2) ongoing review (>1/year) of the data. A potential PM for physicians performing EUS and EBUS FNAs is the number of procedures resulting in unsatisfactory diagnoses, as these cases lead to additional/repeat procedures increasing exposure to risks/morbidities, patient anxiety, and increasing costs. The aim of this study is to assess the utility of adequacy rate (AR) (satisfactory vs unsatisfactory) as a PM for OPPE in physicians who perform EUS/EBUS guided FNAs.
Design: A retrospective review of the electronic medical record was performed in our institution for EUS and EBUS FNAs (2 and 5 years respectively). Physician performance was measured by their AR. We also reviewed the number of passes performed for each procedure.

Results: A total of 5 physicians performed a range of 17 to 160 (mean=59) EUS or EBUS FNA procedures. The study included 296 cases, 219 EUS and 77 EBUS. EBUS FNAs on average were performed less frequently than EUS FNA and at more irregular time intervals.

Physicians performing EUS or EBUS

Physician	Number of cases	Number with adequacy assessment	Type	Avg number passes/ procedure	AR (%)
A	39	39	EBUS	N/A	74
B	21	21	EBUS	N/A	57
C	17	17	EBUS	N/A	23.5
D	160	103	EUS	3.4	86
E	59	30	EUS	3.8	74.6





Conclusions: We demonstrated that utilization of AR is a useful, simple, low cost tool for PM in OPPE of physicians performing EUS/EBUS FNAs. The number of passes did not affect the satisfactory rate. Additionally, more frequent and/or stringent ARs can be tracked for physicians performing below a set standard satisfactory rate for OPPE and participating in Focused Professional Practive Evaluations (FPPE).

338 Implementation of the Bethesda System for Reporting Thyroid Cytopathology: Observations from the 2011 Thyroid Supplemental Questionnaire of the College of American Pathologists

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Background: Although information about The Bethesda System for Reporting Thyroid Cytopathology (TBSRTC) has been widely disseminated since its inception in 2007, the degree of its implementation and impact on daily practice has not been formally evaluated. The objective of this study was to assess the extent of uptake of TBSRTC across pathology laboratories and to evaluate its impact on daily practice by collating participant responses to the 2011 supplemental thyroid questionnaire of the College of American Pathologists (CAP).

Design: A questionnaire was designed to gather information about various aspects of the TBSRTC and mailed in June 2011 to 2063 laboratories participating in the CAP cytopathology interlaboratory comparison program. The participating laboratories' answers were collated and analyzed.

Results: 741 laboratories returned the survey (36%). While 60.9% (N=451) and 17.1% (N=127) of laboratories reported using TBSRTC or planning to use it in the near future, respectively, 22% (N=163) had no plans to implement TBSRTC. Of the latter, 32% (N=70) stated that they were unaware of this classification. The majority (78.3%, N=343) of the laboratories use TBSRTC as published while 21.7% (N=95) use it with minor modifications. Most report that the use of TBSRTC has caused either no change (N=67, 15.2%) or only minor changes (N=353, 80.2%) in the terminology and diagnostic criteria previously used in their laboratories.

Conclusions: According to the data collected by the 2011 CAP thyroid supplemental questionnaire, TBSRTC is generally well implemented in North American pathology laboratories. However, because some participants are not aware of this new classification, further dissemination of TBSRTC is warranted.

339 Endobronchial Ultrasound-Guided Fine-Needle Aspiration Cytology, a More Efficacious Diagnostic Modality to EBUS-Core Needle Biopsy – The Washington University Experience

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Background: Although, mediastinoscopy and CT-guided biopsy remain the gold standard procedure for assessing mediastinal lymph nodes and pulmonary lesions, Endobronchial Ultrasound-Guided Fine-Needle Aspiration (EBUS-FNA) has emerged as a quintessential less invasive tool for accessing and cytologic evaluation of mediastinal lymph nodes and pulmonary masses for the purpose of cancer staging and primary diagnosis, respectively. At the Washington University School of Medicine, immediate cytologic assessment and diagnosis of EBUS-FNA is done by telepathology. We compare the diagnostic yield of EBUS-FNA cytology to EUBS-core needle biopsy (CNB).

Design: We performed a retrospective cohort study by reviewing and comparing the electronic medical record database of EBUS-FNA and EBUS-CNB, (histologic) diagnoses of all patients at our institution who underwent EBUS-FNA procedure between December 12, 2010 to August 10, 2012. A total of 476 patients were used in this study. Of these, 224 patients had concurrent core biopsies.

Results: Of the 476 cases examined, the mean age was 62+/-14 years with 53% being males. In 249 out of the total cases, the less invasive FNA aspiration technique alone produced enough diagnostic cytology material removing any need for concurrent tissue core biopsy. Of the 249 cases, 164 were diagnosed as malignant tumors. As expected, the diagnostic yield of EBUS-FNA cytology was similar to EBUS-CNB (95% compared to 94%, respectively). However, there was discordant diagnosis between cytology and histology in 19 of 227 cases who had both EBUS-FNA and EBUS-CNB done concurrently (~8.4%). Of the discordant diagnostic cases, 12 were correctly diagnosed as malignant by cytology but negative for malignancy by CNB. Four of the 19 discordant cases were called negative for malignancy by cytology and but proved to be malignant by histology. Another two of the 19 discordant cases were diagnosed as granulomatous inflammation by histology but were not picked up by cytology. The last of the 19 cases was diagnosed as malignant by both methods but as a totally different tumor (metastatic squamous cell versus metastatic urothelial cell carcinoma).

Conclusions: EBUS-FNA cytology is more efficacious, less invasive, and shows high-yield accurate diagnosis for mediastinal lymph node and pulmonary lesions as compared to EBUS-CNB.

340 A Superior Technique for Cell Block Preparation for Fine Needle Aspiration

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Background: Cell blocks (CB) from fine needle aspiration biopsies (FNAB) are useful for diagnosis and ancillary studies. Variability in CB techniques can affect diagnostic quality. We describe 3 techniques for CB preparations and performed a statistically validated comparison to determine which system reliably produced the highest quality CB.

Design: We compared 3 CB techniques: 1) The FNAB is rinsed in saline (SR), centrifuged to a pellet, the supernatant is removed and plasma and thrombin are added to form a clot which is submitted to histology. 2) The FNAB needle is rinsed in formalin, centrifuged to a pellet, the supernatant is removed and Histogel™ (HG) is added to form a clot which is submitted to histology. 3) The FNAB is rinsed in formalin, transferred to a test-tube lined by collodion™ polymer, which forms a "collodion-bag" (CLB) membrane, the sample is centrifuged, the bag is removed and tied with a string around the resulting pellet. All samples were submitted to histology in cassettes for routine processing. Four FNAB were performed with standardized technique, on 35 random gross surgical pathology specimens: 1 for direct smears to assess the overall cellularity, and 1 for each of the 3 CB techniques. Blinded review of H&E stained CB slides was performed by 2 pathologists (RB, JO). Each CB slide was scored for cellularity, preservation and architecture, on a scale of 1-3, and the overall best CB was identified. Standard of deviation (SD) and p values were calculated for each category.

Results: Surgical pathology specimens included benign tissue, carcinomas, sarcomas and lymphomas. Mean cellularity score for SR was 1.71 (SD=0.89), for HG was 1.68 (SD=0.67) and for CLB was 3.0 (SD=0). Mean preservation score for SR was 1.31 (SD=0.58), for HG was 1.54 (SD=0.70) and for CLB was 2.91 (SD=0.37). Mean architecture score for SR was 1.45 (SD=0.70), for HG was 1.43 (SD=0.60) and for CLB was 2.71 (SD=0.57). There was no statistical significance between SR or HG when compared for each category. CLB was superior to both SR and HG when compared for each category (p<0.05). The overall best CB was obtained with CLB in 33/35 (94%), with SR in 1/35 (3%) and with HG in 1/35 (3%).

Conclusions: CLB is superior for CB preparation and yields greater cellularity, preservation and architecture in most cases. Our samples were obtained from bench specimens; however standardization in the FNAB technique suggests these results may be extrapolated to patient samples.

341 Evaluation of Small Kidney Masses in the Era of Tumor Ablation Therapy: How Accurate Is Rapid Cytological Diagnosis and Should Separation of the Diagnostic and Treatment Procedures Be Considered?

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Background: As ablative therapies assume a greater role in the management of small renal masses, accurate diagnosis of these lesions becomes increasingly important. At many institutions, including ours, ablation is performed immediately following rapid interpretation of fine needle aspiration biopsy (FNAB) of the mass. This study was undertaken to better understand the value of rapid interpretation performed in this setting, to compare its accuracy and correlation with the final diagnosis, and determine the percentage of patients who undergo treatment regardless of having a benign or non-definitive diagnosis in this setting.

Design: A 7-year retrospective review was performed to identify patients with small solid renal masses who underwent Ultrasound or CT guided FNAB immediately prior to percutaneous radiofrequency or cryoablation. The clinical and radiological characteristics, rapid cytopathologic interpretations by on-site cytopathologists or cytopathology fellows, number of passes performed, and final cytological diagnoses were recorded and analyzed.

Results: A total of 91 patients were identified. Tumor size ranged from 1 to 6.6 cm (mean 2.6 cm). The number of passes ranged from 1-4 (mean 2). At rapid interpretation, FNAB samples were satisfactory in 57 (63%), unsatisfactory in 24 (26%), and indeterminate in 8 (9%) of cases. Two cases lacked an adequacy statement. A definitive final diagnosis was obtained in 46 (80%) samples initially interpreted as satisfactory, 12 (50%) samples initially interpreted as unsatisfactory, and 4 (50%) samples initially interpreted as indeterminate. In 21 (23%) cases the final diagnosis was benign. Overall, a non-definitive final pathologic diagnosis was made in 27 (30%) of patients, and this was primarily due to insufficient material. A cytological diagnosis was provided at the time of rapid interpretation in 66 cases, 36 (55%) of which matched the final diagnosis.

Conclusions: In this setting, although on-site rapid cytopathologic diagnosis is less accurate than final diagnosis, performance of rapid assessment for sample adequacy plays an important role in increasing the rate of a definitive final diagnosis. Given that under the current approach a substantial number of patients with benign or indeterminate final diagnosis undergo ablation, modification of the current approach to separate the diagnostic and therapeutic procedures into separate settings should be considered if the diagnosis would alter clinical management.

342 Comparison of Single vs. Concurrent Multiple HPV Genotypes Infection Pattern in Cervical Cytology of Women with H-SIL

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Background: The contribution of human papillomavirus (HPV) types to the carcinogenesis of cervical cancer has been established for a long time. However, the role of phylogenetically related HPV genotypes and rare variants remains uncertain, as well as the influence of concurrent multiple HPV genotypes infection. The present study aims at studying the pattern of prevalence of several HPV genotypes infecting women with single vs. concurrent multiple HPV genotypes infection with a H-SIL diagnosis in a cervical cytology.

Design: It was conducted a cross-sectional study using Thin-Prep® liquid-based cervical cytology specimens with the diagnosis of H-SIL, in which HPV genotype was sequentially tested. Women with the diagnosis of H-SIL and vaginal specimens or unavailable genotyping results were excluded. Genotypes were determined with a PapilloCheck® system (Greiner Bio-One, Frickenhausen, Germany), a DNA-Chip for the type-specific identification of 18 high-risk and 6 low-risk types of HPV.

Results: Between 2008 and 2011, a total of 1394 liquid-based cervical cytology samples were analysed and the diagnosis of H-SIL made in 207 (14.85%; 95%CI: 13.08-16.82) specimens. Of these, 176 (85.02%; 95%CI: 0.79-89.29) had available and positive HPV genotyping result. Mean age of these women was 39.35 years old (95%CI: 37.81-40.85), being HPV16 the most prevalent genotype (48.30%; 95%CI: 41.03-55.64) followed by HPV31 (14.20%; 95%CI: 9.75-20.18). HPV18 was detected in 5.11% (95%CI: 2.58-9.56) of the samples. Concurrent multiple HPV genotypes were detected in 36.72% (95%CI: 29.55-43.89) of the samples. The prevalence of the nine most common HPV genotypes detected varied significantly according to the presence of single vs. concurrent multiple HPV genotypes (p=0.016). Moreover women with concurrent multiple HPV genotypes were on average 3.53 (95%CI: 0.43-6.64) years younger than women with single genotype infection.

Conclusions: Our results suggest that women with multiple vs. single genotype HPV infection differ in terms of age and distribution of the most prevalent HPV genotypes. Moreover, we provide further evidence of the predominance of HPV16 in H-SIL lesions of the uterine cervix.

343 Digital Holographic Microscopy: Screening Tool for Cervix Cancer. Preliminary Study

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Background: Liquid-based cytology Pap test (LBC) replaces the conventional Pap test as LBC is more efficient, partly owing to computer assistance. However the diagnosis still depends on human eye, sensitivity is not perfect, and LBC requires complex technology. Holocyt Intelligence System Corp. (HIS) develops of a digital holographic microscopy (DHM) instrument and software, which provides a three dimensional image reconstruction of uterine cervical cells, without delay nor staining. The purpose of this preliminary study was to identify reproducible optic criteria of cells considered normal or abnormal.

Design: Analysis was performed with the DHM (model c-Line), vial "c-Box", computer and software (Ovizio®) lent by HIS). Residual material from the three labs, randomly chosen, was analysed after LBC (Thinprep® (UHA) and Turbitec® (IJG, PCA). Residual material poured into a "c-Box" was subjected to DHM. At least 20 "normal" or "abnormal" looking cells reconstructed by software were selected per vial. The following were automatically measured and extracted: maximum height nuclear (MHN), normalized nuclear to cytoplasm height (NHN), nuclear diameter (ND) and nucleo-cytoplasmic ratio (NCR). Low and high grade cells were grouped, due to small samples figures. Values are expressed as mean ± SD. Statistical analysis of the data were performed using Mann–Whitney and ROC curve. Differences are considered significant when $p < 0.05$.

Results: In 32 specimens (12 in UHA, 15 in IJG, 5 in PCA) with normal Pap test, 1333 cells analyzed with DHM. In 21 specimens (12 in UHA, 4 in IJG, 5 in PCA) with abnormal Pap test, 494 cells were analyzed with DHM. Available histologic correlation was: 8 CIN1 (6 LSIL, 2 ASC-US), 2 CIN3 (1 LSIL, 1 ASC-H), 3 normal. with pooled three or one observer (except for MHN in PCA p=0.56), a significant difference between normal and abnormal specimens was clear (three observers: NHN:0.45±0.14 vs 0.59±0.21, MHN:0.19±0.08 vs 0.28±0.12, ND:9.13±2.2 vs 18.6±9.8, NCR:0.04±0.04 vs 36±0.32). Globally, the areas under the ROC curves (95% confidence intervals) are 0.82[0.79-0.84] for NHN, 0.85[0.82-0.87] for MHN, 0.94[0.93-0.96] for ND, 0.97[0.96-0.98] for NCR.

Conclusions: This preliminary study demonstrates that the criteria analyzed with DHM had good area under the ROC. We feel encouraged to perform large scale validation study. Hopefully holograhic analysis will be performed automatically, will provide quickly clear delineation of normal and abnormal cells, on a closed vial, without alteration of fixed cells, allowing ancillary studies.

344 Can Tall Cell Microcarcinoma Be Diagnosed on Fine Needle Aspiration Cytology? An Analysis of Clinicopathological Features, Preoperative Fine Needle Aspiration and Genetic Alteration

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Background: Thyroid papillary microcarcinomas are by definition equal to or less than 1.0 cm in size. These are often considered incidental and clinically insignificant. Tall cell variant of papillary thyroid carcinoma is a rare variant that is associated with a more aggressive behavior and often presents at an advanced stage. Recently we described the tall cell variant of papillary thyroid microcarcinoma (microTCV). Despite their small size, microTCV displayed aggressive pathological features. This study was designed to determine if microTCV have features sufficiently characteristic for their preoperative diagnosis as this would help to plan an appropriate surgical strategy.

Design: microTCV cases over the last 10 years were identified from our files. The cytology slides were reviewed and the findings were correlated with clinicopathologic features and with results of BRAF V600E mutation analysis.

Results: Twenty-one cases were identified (18 females and 3 males). The average age of patients was 53 years (range, 34-73 years). All patients underwent total thyroidectomy. Thirteen of the thyroids contained multifocal microTCV. Tumor size ranged from 0.2 cm to 0.9 cm (mean, 0.70 cm). Background lymphocytic thyroiditis was present in 10 cases. At presentation 29% had lymph node metastasis, vascular invasion was present in 19% while extrathyroidal extension was present in 38% of cases. Fifty eight percent of cases had smears with papillary groups while 68% showed cohesive flat sheets. The frequency of cytologic features were as follows: nuclear grooves – 90%; abundant dense eosinophilic cytoplasm – 90%; nuclear enlargement – 74%; nuclear pseudoinclusions – 74%; irregular nuclear membranes – 47%; powdery chromatin – 42%; crowding and overlapping – 42%. Twenty cases (95%) were positive for BRAF V600E mutation.

Conclusions: microTCV is frequently associated with multifocality, extrathyroidal extension, and lymph node involvement at presentation and high BRAF mutation rate, hence the need for recognition on FNA. Features with high diagnostic yield include cohesive flat sheets, abundant dense eosinophilic cytoplasm, nuclear enlargement and grooves, among others.

345 The Utilization of Stained Cytologic Direct Smears for ALK Rearrangement Testing of Lung Adenocarcinoma

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Background: Advances in our understanding of the molecular landscape of non-small cell lung cancer (NSCLC) has provided opportunities for targeted therapeutics. Rearrangements involving the *ALK* gene are present in approximately 5% of NSCLC. Crizotinib has been recently approved for the treatment of NSCLCs harboring *ALK* rearrangements. A large number of patients with lung cancer are diagnosed at high stage and are not candidates for surgical resection of their primary tumors. For these patients, cytologic specimens often represent the only tumor samples available for tissue diagnoses and necessary molecular studies. Cell block (CB) preparations are routinely used for molecular studies. However, insufficient cell block cellularity, in some instances, can impede the performance of these assays.

Design: Paired Diff-Quik stained smears and corresponding CB sections from 23 cytology cases of lung adenocarcinoma were analyzed by fluorescence in-situ hybridization (FISH) for *ALK* rearrangement. Prior to testing, the smears were examined to identify tumor cell enriched areas that were marked using a diamond-tipped scribe, decoverslipped in xylene, and then destained in acid-alcohol.

Results: Paired *ALK* rearrangement analysis by FISH was successful for all 23 cases. An *ALK* rearrangement was detected on direct smears and CB sections in 4 (17%) and

3 (13%) of 23 cases, respectively. Concordant FISH results for smears and CBs were observed in 22 (96%) of 23 cases. In the one discordant case, an *ALK* rearrangement was detected on the direct smear but not in the CB. RT-PCR analysis of this CB revealed the presence of an *EML4-ALK* rearrangement, confirming a false negative FISH result in the CB section.

Conclusions: Stained cytologic direct smears can be effectively utilized for *ALK* rearrangement analysis by FISH. This approach represents a useful safeguard when insufficient CB cellularity is encountered. Based on our experience detailed herein in conjunction with prior reports regarding the utilization of direct smears for other molecular applications, complementary methods of cytologic specimen triage for molecular testing will be presented. An overall triage mechanism that incorporates safeguards has the potential to prevent repeat procedures, for which sufficient CB cellularity is not necessarily guaranteed, and prevent delays in treatment in this era of precision medicine.

346 Rapid On-Site Cytologic Evaluation (ROSE) of Endoscopic Ultrasound-Guided Fine Needle Aspirations (EUS-FNAs): A Tale of Two Institutions

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Background: ROSE is generally considered to improve diagnostic yield of EUS-FNAs. We investigated the use of ROSE on EUS-FNA procedures at two academic medical centers ("MCA" and "MCB"). At MCA, ROSE is rarely performed on EUS-FNAs, while ROSE is performed on almost all EUS-FNAs at MCB. The purpose of this study is to determine the impact of ROSE on EUS-FNA outcome by comparing experiences at MCA and MCB.

Design: A retrospective review of all EUS-FNAs accessioned between 8/2011-8/2012 at MCA and MCB was performed. Electronic medical records were reviewed for performance of ROSE, procedure time, number of repeat procedures, and final diagnoses. Statistical significance of difference was determined by t test for continuous variables and by chi-square for categorical variables.

Results:

Data

Variable	Institution		P-value
	MCA	MCB	
Procedure #	302	114	
Repeat procedures	31 (10%)	5 (4%)	0.06
Patient #	271	109	
Lesion Sampled			<0.001
Pancreas only	159 (53%)	94 (82%)	
Lymph nodes only	111 (37%)	12 (11%)	
Both	31 (10%)	8 (7%)	
ROSE done	16 (5%)	106 (93%)	<0.001
Average procedure time in minutes	37 (2-80)	53 (22-137)	<0.0001
Average procedure time if pancreas only was sampled	36 (2-80)	51 (22-127)	<0.0001
Average procedure time if lymph node only was sampled	38 (13-67)	60 (42-137)	<0.0001
Average procedure time if both were sampled	40 (22-80)	66 (47-89)	0.0002
Average procedure time if 1 site was sampled	37 (2-80)	51 (22-137)	<0.0001
Average procedure time if ≥ 2 sites were sampled	40 (22-80)	66 (37-89)	<0.0001
Final diagnosis of each procedure			<0.001
Non-diagnostic/negative	124 (42%)	19 (17%)	
Atypical	42 (14%)	3 (3%)	
Suspicious	25 (8%)	2 (2%)	
Positive	111 (37%)	90 (79%)	

While only 5% of all procedures at MCA underwent ROSE, 69% of MCA's ROSEs were performed on repeat procedures (35% of repeat procedures at MCA underwent ROSE). However, upon repeat procedure at MCA, the positive rate was approximately the same as the overall rate (35% vs 37%). The positive rate of those procedures with ROSE at MCA was 44% vs 36% without ROSE (P = 0.55). This indicates that the relatively low overall positive rate at MCA (37%) is not likely due to lack of ROSE.

Conclusions: Although it is unclear whether ROSE significantly affects diagnostic yield, ROSE appears to significantly increase procedure time of EUS-FNA but is associated with fewer repeat procedures. The overall cost effectiveness of ROSE needs further investigation to determine if lower repeat rates are an adequate trade-off for significantly longer procedure times and personnel commitment.

347 Fine Needle Aspiration Biopsy of Hodgkin Lymphoma: The UCSF Experience

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Background: Fine needle aspiration biopsy (FNAB) is the initial diagnostic procedure of choice for unexplained lymphadenopathy. FNAB, in combination with flow cytometry and other adjunct testing modalities, is highly reliable in the detection of mature B-cell lymphomas and mestastatic neoplasms. The accuracy of FNAB for the diagnosis of Hodgkin lymphoma (HL) is reported to be approximately 33-92%; thus, the role of FNAB in primary diagnosis and management of patients with HL remains controversial. Our study reviews the performance of FNAB in the diagnosis of HL at UCSF over the past 15 years.

Design: The UCSF Department of Pathology database was searched for all lymph node excisional biopsy specimens where a diagnosis of HL was rendered between 1998 and 2012. All cases where FNAB of the same node or node group was performed preoperatively were selected for diagnostic correlation. A separate search of all lymph node FNAB specimens with a diagnosis of HL was performed. Data regarding the use of confirmatory immunohistochemical stains and clinical followup were obtained. The diagnostic sensitivity and false negative rate were calculated based on the assembled data.

Results: Twenty-five patients were identified who had a lymph node excisional biopsy specimen with a diagnosis of HL with a preceding FNAB of the same node or node group. Definitive diagnosis of HL was rendered on the preceding FNAB specimen in 11 cases. FNAB samples were insufficient for diagnosis in 4 cases. Using surgical biopsy as the "gold standard" for diagnosis of Hodgkin lymphoma, FNAB has a sensitivity of approximately 85% and a false negative rate of 8%. An additional 15 patients received a definitive diagnosis of HL on FNAB and went directly to therapy, avoiding surgical biopsy. If these patients are included in this calculation, the sensitivity increases to 93% and the false negative rate falls to 7%. False negative cases include one CT-guided FNAB with concurrent core biopsy and one palpation-guided FNAB.

Conclusions: Despite the relatively small number of cases for which a primary diagnosis of HL is rendered, primary diagnosis of HL by FNAB is highly reliable at our institution. No false positive diagnoses were identified over a 15 year time interval. The sensitivity of this test is >85% and diagnosis is frequently supported by appropriate immunohistochemical stains.

348 Touch Preps of Core Needle Biopsies from Renal Masses: 5 Years Retrospective Review and Interpretation Pitfalls

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Background: Due to increased use of radiologic studies, incidental renal cell carcinoma is more frequently detected. Minimally invasive techniques like percutaneous radiofrequency ablation are available for treatment of high risk patients and require documentation of presence of a neoplasm by CT-guided core needle biopsies. Touch preps of core needle biopsies are performed to evaluate adequacy of specimen collection and documentation of a malignant neoplasm allows interventional radiologists to perform radiofrequency ablation in a single procedure.

Design: We reviewed our electronic records from Jan. 2008-Apr. 2012 and identified 60 cases of CT-guided biopsies of renal masses on which intra-operative touch preparations were performed. Diff-Quik and rapid H&E stained smears were examined by cytopathologists blinded to the original interpretation or results of permanent sections and correlated to final biopsy diagnosis or follow-up where available.

Results: The series included 46 malignant renal neoplasms (renal cell carcinoma, oncocytic neoplasm, mixed epithelial and stromal tumor and urothelial carcinoma), 3 metastases from lung carcinomas, 2 angiomyolipomas and 12 benign inflammatory processes. Interpretation of intraoperative touch preps correctly identified the lesion as neoplastic or not in 47 of 60 cases (78.3%). Six cases were interpreted as neoplastic on touch preps, however, permanent sections of the core biopsy were benign (false positive rate of 10%) and 7 cases were called non-neoplastic on touch preps with a malignant neoplasm demonstrated on follow-up (false negative rate 11.6%). Angiomyolipoma, oncocytic change in renal tubular epithelium and infarcted parenchyma were the most frequent causes of false positive interpretation, whereas sparse cellularity and sampling were the main reasons for false negative results.

Conclusions: Touch preps of CT-guided core needle biopsies from renal lesions are imperfect tools in predicting malignant neoplasms. Knowledge of the limitations in interpretation of these samples allows the radiologist to take a more informed decision regarding performing radiofrequency ablation in a one versus two step procedure.

349 When Should We Send a Cellular Lymphoid Effusion for Lymphoma Work-Up

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Background: Many malignant effusions caused by hematopoietic malignancies often emerge as the initial presentation, especially low-grade lymphomas, such as chronic lymphocytic leukemia/lymphoma, mantle cell lymphoma, marginal zone cell lymphoma and low-grade follicular lymphoma in elderly patients. These low-grade lymphomas consist of relatively small lymphocytes, cytologically difficult to distinguish from benign lymphocytes. Definitive diagnosis of lymphoma in body fluids is inevitably based on further ancillary immunohistochemical studies, flow cytometric analyses and sometimes molecular tests to detect clonality. Triage of effusion specimens with atypical lymphoid populations for further lymphoma work-up will not only render a definitive diagnosis but may also reduce unnecessary procedures and initiate prompt and appropriate treatment. In this study, we review 40 lymphoma cases with neoplastic effusions and summarize the cytologic features that potentially trigger further work-up to diagnose non-Hodgkin's lymphoma.

Design: Thin-prep slides from10 cases of CLL, 4 cases of mantle cell/marginal zone lymphoma, 4 cases of low grade follicular lymphoma, 3 cases of T cell lymphoma, 10 cases of large cell lymphoma, 3 cases of T cell lymphoma and 9 cases of benign inflammatory effusion were reviewed. The following nuclear features were evaluated as abnormal if seen in >30% of lesional cells: enlarged nuclei (>medium size), cleaved nuclei, clumped chromatin pattern, prominent nuclear membrane, nuclear lobulation, distinct nucleoli (any size), and mitoses.

Results: On Thin-prep slides, distinct nucleoli, of any size, were the most frequently encountered features in all lymphomas. Cleaved nuclei and clumped chromatin pattern were also very common in lymphomatous effusions (as shown in Table).

Table: Nuclear features of lymphocytes from different lymphoid effusion.

	Enlarged nuclei	Cleaved nuclei	Clumped chromatin	Prominent nuclear membrane	Distinct nucleoli	Nuclear lobulation	mitosis
Chronic lymphocytic lymphoma (10)	4/10	7/10	8/10	8/10	2/10	0/10	4/10
Mantel cell/marginal zone cell lymphoma(4)	2/4	3/4	3/4	3/4	4/4	2/4	0/4
Follicular lymphoma (4)	3/4	3/4	3/4	3/4	4/4	1/4	3/4
Large cell lymphoma(10)	10/10	10/10	4/10	5/10	10/10	5/10	3/10
T cell lymphoma(3)	3/3	3/3	1/3	1/3	3/3	0/3	0/3
Benign lymphoid effusion (9)	0/9	0/9	0/9	0/9	0/9	0/9	1/9

Conclusions: The presence of distinct nucleoli in a cellular lymphoid effusion from elder patients should alert the cytopathologist to examine the clinical history and initiate further diagnostic work-up to rule in/out lymphoma involvement.

350 Application of the Bethesda Thyroid System in the Pediatric Population

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Background: Few studies have analyzed the Bethesda System for reporting pediatric thyroid nodules since the application of this system in 2007. Likewise, the usefulness of molecular testing in the pediatric age group has been reported in few studies. This study aims to evaluate the usefulness of the Bethesda System in pediatric thyroid nodules as well as the utility of molecular testing.

Design: All thyroid fine needle aspirations (FNAs) from patients 21 years of age or younger were collected from the pathology files of a tertiary referral hospital between the years 2008-2012. The cytologic diagnoses were reviewed and retrospectively correlated with molecular testing results and histologic results in cases with surgical follow-up. Nearly all FNAs were performed under ultrasound guidance.

Results: 234 FNAs from 225 patients were identified, accounting for 1.8% of all thyroid FNA evaluated during the study period. Among the 234 FNAs, 69(29%) cases had histologic follow up and 17 of 69 (25%) cases had BRAF analysis. The cohort included 183 (80%) females between 12 and 21 years of age (mean 17± 3.2) and 42 (20%) males between 4 and 21 years (mean 17± 3.1). Cytologic diagnoses were as follows: 156 (66.7%) cases benign, 10 (4.3%) cases AUS (atypia of of undetermined significance), 10 (4.3%) cases follicular neoplasm, 12 (5.1%) cases suspicious for malignancy, 35 (15.0%) cases malignant and 11 (4.7%) cases unsatisfactory. Of the 69 cases with histologic follow up, 23 (33%) cases were non neoplastic, 7 (10%) cases were benign neoplasms (adenoma), and 39 (56%) cases were malignant.

Cyto-Histology Correlation

	Benign	AUS	FN	Suspicious	Malignant
Benign	18	3	1	1	0
FN	3	0	4	0	0
Malignant	0 (0%)	4 (57.1%)	1 (16.7%)	7 (87.5%)	27 (100%)

Of the 17 cases sent for mutational analysis, 10 (59%) cases were positive for the BRAF mutation. All malignancies with the BRAF mutation were papillary thyroid carcinoma at final diagnosis. The sensitivity and specificity of diagnosing thyroid malignancy in this pediatric cohort is 100% and 94.7%, respectively.

Conclusions: Thyroid FNA is rarely encountered in the pediatric population; however, it is a highly sensitive and specific method for evaluating thyroid malignancy. There is a higher incidence of malignancy (15%) in children compared to adults (5.5%). The increased incidence of malignancy in this study may in part be due to a population bias, being drawn from a tertiary referral center. The prevalence of BRAF mutation in this cohort was similar to that in adults. This is in contrast to previous studies on pediatric thyroid nodules which reported a lower incidence of BRAF mutations in children.

351 The Role of p40 Immunostain in the Cytological Differential Diagnosis of Squamous Cell Carcinoma and Adenocarcinoma of the Lung

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Background: Subtyping of non-small cell lung cancers (NSCLCs) has profound therapeutic implications. A panel of TTF-1/p63 immunostains is the current recommendation for differentiation of adenocarcinoma (ADC) from squamous cell carcinoma (SCC) in small biopsies or cytological specimens. However, p63 positivity, though seen in virtually all SCC cases, can be seen in as high as 30% ADCs. Furthermore, only up to 70% of ADC cases show positive TTF-1. Recently, p40 has been shown a promising marker in identifying SCC with high sensitivity and specificity on surgically resected tumors. In this study, we evaluated p40 immunoreactivity in the fine needle aspiration (FNA) specimens of lung ADCs and SCCs.

Design: The cytopathology archive was searched for the cases with FNA diagnosis of lung cancers at our institution between January 2008 to July 2012. Only the cases with the diagnosis of either ADC or SCC of the lung on surgical follow-up were included in the study. The p40 immunostain was performed on the cell-block section of FNA specimens. Nuclear staining was semi-quantatively evaluated as 0 (none), 1+ (1-25%), 2+ (25-50%), 3+ (50-75%), and 4+ (75-100%).

Results: A total of 63 cases were identified from 32 male and 31 female patients with ages ranging from 33 to 86 years. The specimens included 26 lung and mediastinal lymph node FNAs with final diagnosis of ADC on surgical follow-up in 34 cases and SCC in 29 cases. Positive p40 immunoreactivity was seen in 26 of 29 cases (90%). Only one of thirty-four ADCs (3%) was positive for p40. Interestingly, two of three SCCs with a negative p40 result were well differentiated keratinizing SCC. The only positive ADC case only had a focal positive p40 stain. The calculated sensitivity and specificity of p40 immunostain for differentiation of SCCs from ADCs were 90% and 97%, respectively.

Table 1. p40 Expression in the Cytological Specimens of Squamous Cell Carcinoma and Adenocarcinoma of the Lung

Surgical Diagnosis	Case #	p40 Immunoreactivity				
	n	0	1+	2+	3+	4+
Squamous Cell Carcinoma	29	3	3	5	6	12
Adenocarcinoma	34	33	1	0	0	0

Conclusions: Our data demonstrate that p40 has high sensitivity and specificity for identifying lung SCCs on the FNA cell-block materials, which might be superior to p63 immunostain in the subtyping of NSCLCs.

352 Fine Needle Aspiration Diagnosis of Mucinous Cystic Neoplasms and Intraductal Papillary Mucinous Neoplasms of the Pancreas: Cytomorphology, CEA Level and K-ras Mutation Status

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Background: Mucinous cystic neoplasm (MCN) and intraductal papillary mucinous neoplasm (IPMN) are the two most common mucinous lesions in the pancreas. Recognition of these lesions preoperatively is important due to their association with variable dysplasia and invasive carcinoma. However, accurate diagnosis could be difficult based on cytomorphologic features alone and may require clinical correlation and ancillary studies. In this retrospective study, we review the results of cytological diagnosis, CEA level and K-ras mutation status in MCNs and IPMNs.

Design: The Cytopathology archives were searched for cystic or solid/cystic pancreatic lesions diagnosed by endoscopic ultrasound-guided fine needle aspiration (EUS-FNA) biopsy at our institution from January 2005 to July 2011. The cases were included in the study only when there was surgical follow-up with diagnosis of either MCN or IPMN. The cytological diagnosis and the results of CEA level and K-ras mutation status, if performed, were retrospectively reviewed.

Results: A total of 61 cases were identified, including 25 MCN and 36 IPMN cases diagnosed on surgical follow-up. The MCN cases had low-grade dysplasia, moderate dysplasia, or high-grade dysplasia/invasive carcinoma in 21, 1, and 3 cases, respectively. Low-grade dysplasia, moderate dysplasia and high-grade dysplasia/invasive carcinoma were present in 17, 10, and 9 IPMN cases. Based on cytomorphologic features, mucinous neoplasm was recognized in 11 of 25 MCN cases (44%) and in 26 of 36 IPMN cases (72%) including a malignant diagnosis in 3 MCNs and 6 IPMNs. Elevated CEA level (>= 192 ng/ml) was seen in 11 of 14 MCN cases (79%) and 6 of 7 IPMN cases (86%) while K-ras mutation was identified in 7 of 13 MCN cases (54%) and 8 of 15 IPMN cases (53%). By combing cytomorphologic features with CEA level and K-ras, the sensitivity for identification of mucinous neoplasm was increased from 44% to 68% in MCNs and from 72% to 83% in IPMN cases.

Table 1. Cytomorphology, CEA Level and K-ras Mutation in Mucinous Neoplasms

Surgical Diagnosis	Cytological Diagnosis			CEA Level		K-ras Mutation
	Negative	Neoplasm	Malignant	<192 ng/ml	>=192 ng/ml	Negtaive	Positive
MCN (n=25)	14	8	3	3	11	6	7
IPMN (n=36)	10	20	6	1	6	7	8

Conclusions: Our data demonstrate that EUS-FNA has a higher sensitivity for identifying IPMNs than MCNs based on cytomorphologic features. Adjunctive ancillary studies such as CEA level and K-ras mutational analysis increase the sensitivity, especially for identification of MCNs.

353 Atypia of Undetermined Significance in Thyroid Fine Needle Aspiration Biopsy Evaluated by Whole Slide Image Analysis with Risk Assessment of Malignancy

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Background: Atypia of undetermined significance (AUS) is a specific category of thyroid FNA biopsy that presents significant interpretive and patient management challenges. The cytomorphologic features are subjective and difficult to apply consistently. Individual and institutional rates of AUS can vary widely from low single digits up to 20%. There is an increased intermediate incidence of malignancy in the AUS category. Defining objective criteria could allow for more uniform and consistent categorization and risk assessment of malignancy of thyroid AUS.

Design: In order to investigate objective morphologic criteria for the classification of thyroid AUS and asses for malignancy risk, whole slide images (WSI) (Aperio) were prepared and analyzed by image analysis for a set of morphologic criteria. For one calendar year, all thyroid FNA AUS cases were identified and those with subsequent thyroid excision selected. Each FNA case was reviewed and a single representative Diff-Quick stained smear selected and subjected to WSI. The WSI were evaluated in a blinded fashion. All individual follicular groups on the WSI were manually delineated, and measured for total group number, group area (um), nuclear area (um), and nuclear to cytoplasmic ratio (N:C).

Results: There were 44 cases of thyroid AUS with surgical excision and defined outcome. Surgical resection categorized 15 benign non-neoplastic, 8 follicular adenomas, and 21 malignant entities. For malignant cases, 17/21 (81%) had an N:C ratio of less than 0.50 and 4/21 (19%) had an N:C ratio of greater than 0.50. For benign non-neoplastic cases, 6/15 (40%) had an N:C ratio of less than 0.50 and 9/15 (60%) had an N:C ratio of greater than 0.50. In thyroid AUS patients, a N:C ratio of less than 0.50 had a risk ratio for malignancy of 2.4. For malignancies, 17/21 (81%) had over 20 cell groups while the benign non-neoplastic category had 8/15 (53%) with greater than 20 cell groups. The benign non-neoplastic category had an average cell group count of 46 compared to 67 for the malignant category.

Conclusions: WSI image analysis can provide objective morphologic measurements. Utilizing WSI with image analysis on thyroid AUS cases with known outcomes demonstrated that an N:C ratio of less than 0.50 had a 2.4 risk ratio for malignancy.

Determining subsequent clinical follow up on patients with AUS is clinically evolving. Establishing defined objective WSI criteria has the potential to provide a risk assessment of malignancy for AUS thyroid FNA cases.

354 Massive Parallel Sequencing To Assess the Mutational Landscape of Fine Needle Aspirate Samples: A Pilot Study

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Background: Nowadays, massive parallel sequencing(MPS)is shaping research in the field of life sciences.This fast evolving technology is starting to move into the clinical diagnostic arena.However,only with the recent introduction of the benchtop sequencers it can be adopted by the majority of molecular pathology laboratories.The possibility of performing genomic studies with small amounts of material obtained,for example,by fine-needle aspiration(FNA),can minimize invasive procedures and allow the monitoring of cancer,including therapeutic response,with repeated testing.

Design: In this pilot study we aim to address the possibility of using this technology for assessing the mutational landscape of FNA samples.Four samples collected from Breast cancer patients were used to isolate genomic DNA.A single tube multiplex PCR amplification designed to detect 739 mutations from 46 oncogenes and tumor suppressor genes was performed and the resulting amplicons sequenced using the Ion Torrent PGM sequencer.

Results: Genomic DNA of good quality was obtained from all samples.This enabled the amplification and sequencing of all targeted regions.High coverage was obtained for all amplicons.The use of the variant caller plug-in from the Torrent Suite v2.2 resulted in the determination of the mutational landscape of the FNA samples.Importantly, mutations in genes that can be therapeutically intervened were identified using this strategy,namely *PIK3CA*, *SMAD4* and *KDR*.This resulted in findings that could be used for a better management of the patients,and that would not be obtained using the standard routine practice.

Conclusions: In this study we present a workflow that provides a comprehensive genetic screening tool for FNA samples, in a fast and cost-efficient manner.This may provide a valuable tool for the management of cancer patients that can be implemented in most molecular pathology laboratories.

355 GATA3 Expression in Cytology Samples of Metastatic Breast Versus Gynecological Carcinoma and Comparison with GCDFP15 and Mammaglobin

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Background: GATA3 (GATA binding protein 3) is a transcription factor reported to play a role in cell proliferation and differentiation. It has been shown to be highly expressed in the Luminal A subtype of breast cancer. In contrast to the data available for GCDFP15 and mammaglobin, there is lack of reports on the utility of GATA3 in distinguishing metastatic breast versus metastatic gynecological cancer in cytology specimens.

Design: 29 cytology cases comprised of 19 effusions and 10 fine-needle aspirations were selected (17 cases of metastatic breast cancer, 9 cases of metastatic ovarian cancer and 3 cases of metastatic endometrial cancer). All cases had surgical resections on which final diagnosis had been made. Immunostaining was performed for GATA3, GCDFP15 and Mammaglobin. For GATA-3 staining intensity was scored as 1+, 2+ and 3+ and an H-score was calculated by multiplying the intensity and the percentage of neoplastic cells that stained. GATA3 expression was compared with that of GCDFP15 and mammaglobin.

Results: All 17 (100%) of metastatic breast cancer cases, including one ER-negative case, stained with GATA3. This was significantly higher than with GCDFP15 (4/17, 23.53%; $p<0.0001$) and mammaglobin (6/17, 35.29%, $p<0.0001$). The mean H-score for GATA3 was 224. None of the metastatic endometrial or ovarian carcinomas were positive for GATA3. GCDFP15 also showed no staining in metastatic endometrial and ovarian carcinomas, while mammaglobin showed immunoreactive cells in 1/ 9 (11.11%) metastatic ovarian carcinomas.

Conclusions: 1. GATA3 is significantly more sensitive than GCDFP15 and mammaglobin in the detection of metastatic breast cancer in cytology specimens. 2. Staining with GATA3 appears to be specific for a breast primary compared to metastatic carcinomas of gynecologic origin. Thus, in metastases of unknown primary particularly those that are estrogen receptor positive, GATA3 can be valuable in determining the breast origin and excluding a gynecologic primary.

356 The Utility of GLUT-1, CD146 and KOC Immunostains in Distinguishing Malignant Mesothelioma and Reactive Mesothelial Cells from Pleural Effusion

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Background: Pleural effusion is an effective way to detect malignant mesothelioma (MM). However, it is always a cytologically diagnostic challenge to differentiate malignant mesothelial cells from reactive mesothelial cells (RMC). Several recent studies have indicated that GLUT-1, CD146 and KOC (IMP3) are useful immuno-markers for distinguishing MM from RMC. However, these markers were studied separately. Only one comparative analysis was reported and done in surgical specimen. The aim of this study is to evaluate the cytologic utility of GLUT, CD146 and KOC as a diagnostic panel of markers in differentiating MM from RMC in pleural fluid specimen.

Design: We performed a retrospective search for MM diagnosed from pleural effusion specimen in our institution. A total 24 MM cases were found over past 7 years. Because of legal issue, only 8 cases were available for this study. 24 cases of benign pleural effusion with reactive mesothelial cells were selected. All diagnoses were confirmed by tissue biopsy and also support by clinical history and radiology. Immunohistochemical staining for GLUTA-1(355A-16, Cellmarque), CD146 (AC-0052, Epitomics) and KOC (M3625, DAKO) were performed on cell block sections. The stain was recorded as positive when more than 5% of tumor or mesothelial cells were stained.

Results: Of 8 MM cases, 7 were positive for GLUT-1; 6 positive for CD146 and 5 positive for KOC. The 1 GLUT-1 negative MM case is positive for CD146. All RMC cases are negative for CD146 and KOC. Only 1 of 24 RMC case showed focal weakly GLUT-1 stain. As a diagnostic panel for MM, GLUT-1, CD146 and KOC showed sensitivity 87.5%, 75% and 62.5%, respectively, and specificity 95.8%, 100% and 100%. The staining results are summarized in Table 1.

Table 1. Immunostaining results

	GLUT-1	CD146	KOC
MM	7/8	6/8	5/8
RMC	1/24	0/24	0/24

Conclusions: Our limited data indicated that GLUT-1, CD146 and KOC are very useful immuno-markers for distinguishing MM and RMC in cell block sections from pleural effusion. When use the combination of GLU-1 and CD146, 100% sensitivity and 100% specificity for differentiate MM from RMC can be achieved. In our results, KOC showed very high specificity, its sensitivity is lower than reported data. However, its positivity also adds support for the diagnosis of MM. Further studies using larger number of cases should be conducted to obtain more data of these markers.

357 Cost Paradox in Cercival Cancer Screening in South India: Expensive HPV Is a Better Screening Test Than Less Expensive Techniques: Comparison of VIA, Cytology and HPV Testing

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Background: An estimated 70,000 women die every year in India from cervical cancer. A concerted program for cervical cancer does not exist at the national level. Visual Inspection with Acetic Acid (VIA) is very strongly proposed by several organizations including the WHO based on published studies. In this study, we evaluate the efficacy of VIA based screening and cytology based screening. False positive rates of VIA and cytology were compared against Human Papilloma Virus (HPV) results.

Design: We conducted several camps in the Thiruchirapalli and Thirunelveli districts of Tamilnadu. We report the results from two cohorts. In the first cohort of 386 patients, a cervical smear was collected for HPV testing (Cervista) and Liquid Based Cytology (LBC - Thin Prep). In the second cohort of 37 patients, a gynecologist who underwent VIA / colposcopy training at a WHO approved institution, collected specimen for LBC/ HPV followed by VIA / colposcopic examination of the cervix. For VIA based screening, satellite lesions, Lugols Iodine or aceto-white abnormal areas were considered positive. Cytology was positive if the results were ASCUS, LSIL and HSIL.

Results: Result 1. Comparison of LBC with HPV:58 specimens were positive for HPV. Of these 11 were positive by LBC (true positives). There were 33 cases which were positive by LBC, which were negative for HPV (false positive; 8.5% false positive rate among 386 patients). Result 2. Comparison of VIA with HPV: In the second cohort of 37 patients, 7 patients were positive for HPV. Of these 3 patients were positive by VIA. Of the 30 patients, who were negative for HPV, 11 were positive by VIA. All 37 patients were negative by cytology. Based on this the false positive rate of VIA based screening in our set-up is 29.7%.

Conclusions: VIA is increasingly being pushed as a primary screening method in developing countries. However, despite training, there is an unacceptably high degree of false positive (30%) results by VIA. This results in over-treatment in an inordinately large number of patients. The false positivity rate of cytology was 8.5%. Based on the above results, where resources are available, we propose that HPV testing followed by reflex cytology is a better screening strategy than VIA alone. This is applicable to a country such as India, where laboratory based testing and trained cytologists are available.

358 Cytology for the Diagnosis and Surveillance of Upper Urothelial Tract Carcinoma – A Single Cancer Center Experience

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Background: Upper urinary tract (UUT) urothelial carcinoma (UC) is difficult to sample with routine biopsy devices due to the confined anatomy of the renal pelvis and ureter. This study highlights the merits of cytology for diagnosis of UUT UC.

Design: Patients with UUT cytology samples collected at our institution from 01/2009 to 12/2009 were identified from the LIS under an IRB approved protocol. Results of UUT brushings, washings, biopsies, and resection specimens were analyzed. The sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) was calculated for cytology samples. The final diagnosis was established based on biopsy/ resection specimens and clinical follow up.

Results: Thirty-four cases were retrieved (13 brushings, 31 washings) from 31 patients (22 males, 9 females). 13 patients were under surveillance for a prior UC. Table 1 shows the results for all sample types compared to the final diagnosis. Brushings were diagnostic in 12 of 13 cases (Table 2). Washings were diagnostic in all 31 cases (Table 2). For the purpose of statistical analysis, benign and atypical diagnoses were considered as negative and suspicious and malignant diagnoses were considered as positive. In our study, washings (sensitivity = 71%, specificity = 90%, PPV = 94% and NPV = 60%) yielded better results as compared to brushings (sensitivity = 60%, specificity = 50%, PPV = 86%, NPV = 20%). Calculi caused two false positives (1 brushing, 1 washing). Two HG UUTUC had only urine cytology samples which were negative. Seventeen patients had biopsies, and sixteen patients underwent nephroureterctomy/ureterectomy. Clinical follow-up was obtained on all 31 patients.

Table 1. Distribution of Diagnoses rendered by different modalities

Diagnosis	Number	Unsatisfactory	Benign	LG UC	HG UC	CIS	UC NOS
Brushings	13	1	5	-	-	-	7
Washings	31	0	15	-	-	-	16
Biopsy	17	1	1	5	8	0	2
Nephrouretectomy/ ureterectomy	16	0	0	3	12	1	0
Final Diagnosis	34	0	10	5	16	1	2

Table 2. Comparison of Brushings and Washings to Final Diagnoses

Diagnostic Modality	Benign	LG UC	HG UC	UC, NOS
Brushings - Benign/Atypical (n = 5)	1	1	3	0
Brushings - Positive/Suspicious (n = 7)	1	0	6	0
Washings - Benign/Atypical (n = 15)	9	3	3	0
Washings - Positive/Suspicious (n = 16)	1	1	12	2

Conclusions: These results confirm that biopsies are performed less often than washings. Washings performed better than brushings for the diagnosis of UUTUC. Urine cytology alone is not effective at diagnosing UUTUC.

359 Clinical Significance of High Risk Human Papillomavirus (hrHPV) DNA Testing in Women with a Cytological Diagnosis of Atypical Glandular Cells

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Background: Although the casual link between HPV and endocervical adenocarcinoma and its precursors is well established, the role of hrHPV DNA testing as a triage tool for atypical glandular cells (AGC) is still not clearly defined. The objective of this study is to determine if hrHPV testing contributed towards defining histological abnormalities in women with AGC according to preparation types and age groups.

Design: All liquid based Pap tests with the diagnosis of AGC between January 2008 and March 2012 were identified. Patient demographic information, results of hrHPV DNA testing, and subsequent histological follow-up were obtained from our laboratory information system. HrHPV DNA testing was performed using Hybrid Capture II (Qiagen, Gaithersburg MD). Results of histology follow up were divided into 3 groups: Group A: Negative or CIN I; Group B: CIN2+, endocervical adenocarcinoma and AIS; and Group C: endometrial hyperplasia and carcinoma.

Results: A total of 527 Pap tests were diagnosed with AGCs on Pap tests, which account for 0.16% all Pap tests evaluated during the study period. AGC diagnoses accounted for 0.15% of SurePath and 0.19% of ThinPrep; and the difference was not statistically significant. HrHPV status was available in 344 (65.3%) Pap tests; 91 (26.5%) were positive for hrHPV. Tables 1 and 2 summarizes the results of follow-up according to hrHPV status, preparation types, and different age groups (<40 and ≥ 40).

hrHPV Negative Cases

Follow-up	A	B	C	No FU	Total
SurePath	56.8%	3.9%	10.7%	28.6%	206
ThinPrep	81.6%	1.3%	6.6%	10.5%	76
<40 years	47.4%	3.5%	1.8%	47.4%	57
≥40 years	62.8%	3.6%	13.3%	20.4%	196
Total	59.3%	3.6%	10.7%	26.5%	253

hrHPV Positive Cases

Followup	A	B	C	No FU	Total
SurePath	50.0%	42.5%	0.0%	7.5%	80
ThinPrep	54.5%	36.4%	0.0%	9.1%	11
<40 years	50.0%	41.4%	0.0%	8.6%	58
≥40 years	51.5%	42.4%	0.0%	6.1%	33
Total	52.6%	43.4%	0.0%	7.7%	91

Conclusions: The incidence of AGC diagnoses was low for both SurePath and ThinPrep. HrHPV was positive in about a quarter of patients with AGC diagnosis.Less than 4% of patients with a negative hrHPV DNA testing were found to have a significant squamous or glandular cervical lesion.None of the patients with a positive hrHPV status were found to have a significant endometrial lesion.HrHPV DNA testing is useful in triaging patients with a diagnosis of AGC regardless of the preparation types and age groups.

360 Utilization of Cell-Transferred Cytologic Smears in Detection of EGFR and KRAS Mutations on Non-Small Cell Carcinoma of Lung and Comparison to Formalin-Fixed Tissue

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Background: The presence of EGFR and KRAS mutations can be used to predict response to treatment in patients with non-small cell lung cancer (NSCLC). Fine needle aspiration (FNA) can provide cellular material which may be used for such analysis. Formalin-fixed cell blocks (CB) have been used for this purpose; however, CBs sometimes lack adequate cellularity even when the direct smears are highly cellular. The aim of this study is to assess the utilization of the cell transfer technique (CTT) to obtain adequate cellular materials from the direct FNA smears for EGFR and KRAS mutation.

Design: A computerized search of the laboratory information system was performed. FNA cases diagnosed as NSCLC and had previous EGFR and KRAS mutation tests performed on the corresponding CB or biopsies were identified. Tumor cells from both ethanol-fixed Papanicolaou-stained and air-dried Diff-Quik stained direct smears were removed using CTT to submit for EGFR and KRAS mutation testing. The results were compared to those performed on the archived formalin-fixed paraffin embedded (FFPE) corresponding CB (10 cases) and core biopsy (8 cases).

Results: A total of 18 cases including 10 cases for KRAS and 8 cases for EGFR were included in the study. For KRAS, 4 mutations and 5 wild types (WT) showed correlation between CTT and FFPE. In one case, the FFPE showed KRAS mutations while the CTT demonstrated WT result. Re-reviewing the original direct smears from this case showed only scant tumors cells present in a background of abundant benign bronchial cells. EGFR tests demonstrated positive correlation between CTT and FFPE in all 8 cases including 4 mutations and 4 wild types. Both air-dried and ethanol-fixed smears are adequate for the test.

Conclusions: Cell-transferred technique is a feasible method to obtain cellular materials from archived FNA direct smears for EGFR and KRAS mutational tests. There is high agreement rate between the CTT and FFPE tissue. Either air-dried or ethanol-fixed smears can be used successfully for the test. The selection of adequately cellular areas with tumor cells is very important for the correct mutational status.

361 Comparing Automated with Manual HPV ISH, and P16 Immunohistochemistry in Assessing Metastatic Oropharyngeal Carcinoma (OPC)

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Background: HPV-positive tumors are a unique subset of head and neck squamous cell carcinomas (HNSCCs) that are different from HPV-negative tumors in respect to tumor differentiation, genetic changes, risk factors and prognosis. Detection of HPV status is now a standard practice in the pathologic evaluation of HNSCCs. Detection of HPV in metastatic cancer in neck lymph nodes may also be used to localize the primary within oropharynx, with a high degree of certainty. Determining the integration status of HPV by in situ hybridization (ISH) is cost effective and routinely utilized in clinical practice. Strong correlations have been reported between diffuse nuclear and cytoplasmic p16 immunohistochemical staining (IHC) and HPV DNA detection by ISH. In this study, we compared the efficacy of automated HPV ISH utilizing the Enzo probe with manual HPV ISH utilizing the Dako probe, and P16 IHC in the evaluation of metastatic OPCs.

Design: Forty one fine needle aspiration (FNA) cell blocks (CB) from metastatic OPCs were evaluated with automated HPV ISH utilizing the Enzo probe, the manual HPV ISH with the Dako probe, and P16 IHC. HPV ISH was interpreted as positive if a minimum of one tumor cell showed punctate dot like nuclear positivity. P16 was interpreted as positive if 70% of tumor cells showed brown nuclear and cytoplasmic staining. Ten CB from lung squamous cell carcinoma were studied as negative controls.

Results: Thirty of 41 CB (73%) were positive for automated HPV ISH; 25 of 41 CB (60%) with manual HPV ISH. Eighteen of 41 (43%) CB were positive for P16 IHC. The 10 CB from lung squamous cell carcinoma negative controls were all uniformly negative for HPV ISH by both techniques, and P16.

Summary of ISH and IHC results

	HPV ISH (automated)	HPV ISH (manual)	IHC P16
CB	30/41 (73%)	25/41 (60%)	18/41 (43%)

Statistical analysis in CB

	Sensitivity	Specificity	PPV	NPV	Accuracy
HPV ISH (automated)	73%	100%	100%	47%	78%
HPV ISH (manual)	60%	100%	100%	38%	68%
P16 IHC	43%	100%	100%	30%	54%

Abbreviations: PPV, positive predictive value; NPV, negative predictive value

Conclusions: In comparing automated HPV ISH utilizing Enzo probe with manual HPV ISH utilizing the Dako probe, and P16 IHC, we have determined that automated HPV ISH plays a more significant role in determining the HPV status in CB as compared to the other two techniques. P16 staining is easier to recognize and evaluate on tumor cells in contrast to punctate dot like positivity seen in HPV ISH which may be very focal and requires careful evaluation at a higher magnification.

362 Arginase-1: A Highly Specific Marker Separating Pancreatic Adenocarcinoma from Hepatocellular Carcinoma

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Background: The common developmental origin of the adult liver and pancreas has inspired investigation of the potential for hepatopancreatic precursor/stem cell persistence in liver and pancreas. Previous studies have demonstrated that Arginase-1 and HepPar-1 are effective immunohistochemical (IHC) markers for hepatocellular carcinoma (HCC). In this study we explored the possible efficacy of these stains in diagnosing pancreatic adenocarcinoma (PAD).

Design: Arginase-1 and HepPar-1 IHC was performed on formalin-fixed, paraffin-embedded fine needle aspiration (FNA) cell blocks (CB) of PAD (n=46), tissue microarray (TMA) of PAD (n=33), and FNA CB of HCC (n=44). Negative controls were also applied (PAD CB n=7, PAD TMA n=3, HCC CB n=35).

Results: PAD demonstrated Arginase-1 positivity in 0 of 46 cases and HepPar-1 positivity in 7 of 46 (15%) of CB's. PAD TMA demonstrated Arginase-1 positivity in 0 of 33 cases and HepPar-1 positivity in 4 of 33 (12%) of cases. HCC demonstrated Arginase-1 positivity in 37 of 44 (84%) cases and HepPar-1 positivity in 32 of 44 cases (72%).

Analysis of Arginase-1 and HepPar-1 in PAD CB and TMA

	Arginase-1 (CB)	Arginase-1 (TMA)	HepPar-1 (CB)	HepPar-1 (TMA)
Sensitivity	0%	0%	15%	12%
Specificity	100%	100%	100%	100%
PPV	0%	0%	100%	100%
NPV	13%	8%	15%	9%

Analysis of Arginase-1 and HepPar-1 in HCC CB

	Arginase-1	HepPar-1
Sensitivity	84%	72%
Specificity	72%	70%
PPV	74%	69%
NPV	83%	73%

Abbreviations: PPV, positive predictive value; NPV, negative predictive value

Conclusions: Both arginase-1 and HepPar-1 are effective IHC markers of hepatocellular differentiation. Arginase-1 demonstrates superior sensitivity and specificity compared with HepPar-1 in the diagnosis of HCC. However, despite the close relation between the liver and pancreas during embryogenesis, arginase-1 has a low sensitivity and a very high specificity for pancreatic adenocarcinoma. This finding is an important diagnostic tool in separating HCC from pancreatic adenocarcinoma, especially in specimens with small tumor samples.

363 Gynecologic Malignancies Are Commonly Encountered on Fine Needle Aspiration of Supraclavicular Lymph Nodes

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Background: Patient recurrence and survival outcomes have been shown to correlate with stage, most importantly regional lymph node and distant metastasis status, in patients with gynecologic malignancies. Associated with gastric malignancies, the supraclavicular node(s) (SN) are involved in the metastasis of a diverse spectrum of neoplasms, and represent an opportunity to provide nodal staging information on fine-needle aspiration (FNA). Data regarding relative rates of metastasis to this site is limited. We aimed to examine the rate of involvement of the SN on cytology, and to describe useful cytologic features found in observed gynecologic metastases.

Design: A search of the computerized laboratory database was performed for cytologic specimens containing the phrase "supraclavicular" (surgical excisions containing this phrase were included) and "neck" over a two-year period (09/2010 – 09/2012). Results corresponding to aspiration of a lymph node were reviewed, and neoplastic findings recorded and grouped based on site of origin.

Results: We identified 112 malignancies. Proportions are listed in the table below. Gynecologic malignancies were among the most common metastases to the SN (8.9%), trailing only head/neck, pulmonary, and thyroid carcinomas. The gynecologic malignancies identified include squamous cell carcinoma of the cervix (4), uterine endometrioid carcinoma (2), uterine clear cell carcinoma of the uterus (1), uterine carcinoma with clear cell features (1), high grade serous carcinoma of the fallopian tube (1), and juvenile granulosa cell tumor of the ovary (1). These cases were reviewed to determine morphologic features helpful in recognition of the correct diagnosis.

Site of Origin	# of Cases (%)
Head/Neck	47 (41.9%)
Hematologic	16 (14.3%)
Pulmonary	15 (13.4%)
Thyroid	11 (9.8%)
Gynecolgic	10 (8.9%)
Breast	4 (3.6%)
Prostate	3 (2.7%)
Skin	2 (1.8%)
Esophagus	1 (<1%)
Stomach	1 (<1%)
Colon	1 (<1%)
Testicle	1 (<1%)

Conclusions: Gynecologic metastases represent a significant proportion of neoplasms found in SN (8.9%). In the cytologic (or histologic) evaluation of a metastatic carcinoma of unknown origin to the SN, it is important to consider the possibility of a gynecologic origin. These findings indicate advanced disease; therefore inclusion in the differential diagnosis (e.g. in young patients at risk for advanced SCC of the cervix) is paramount. Cytomorphologic and immunohistochemical features of these lesions and correlation with thorough clinical history are critical to reaching a correct diagnosis.

364 Thyroid Bed Fine Needle Aspiration in Patients after Thyroidectomy – A Useful Follow-Up Tool with Proposed Diagnostic Categories

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Background: Thyroid malignancies such as papillary, medullary, and follicular carcinomas are treated by a total or near-total thyroidectomy followed by further ablation of any residual thyroid tissue via radioactive-iodine therapy. Following initial therapy, these patients are closely monitored for recurrence or metastasis. Radiologically-guided fine needle aspiration (FNA) has been used as the appropriate diagnostic modality for occult masses identified radiographically in the thyroid bed. In this study, we report our institutional experience with FNA of the thyroid bed and propose practical diagnostic categories.

Design: A retrospective chart review of all thyroid bed FNAs between April 2008 through January 2012 was performed and a cohort of 28 patients was retrieved. The cytology diagnoses were divided into five categories including: non-diagnostic, inflammatory, bland follicular cells, suspicious for malignancy and malignant. The follow up histologic and/or clinical findings were collected for each category.

Results: The 28 patients included 9 males and 19 females (ages 33-68 years). The prior thyroidectomies were due to papillary thyroid carcinoma (25 cases), follicular carcinoma (1 case), follicular adenoma (1 case), and multinodular goiter (1 case). The thyroid bed FNA diagnosis and follow-up data are shown in Table 1.

Thyroid Bed FNA Diagnostic Categories and Follow-Up Results

Thyroid bed FNA Diagnostic Category	Total Number of cases	No clinical evidence of recurrence/ progression	Surgery performed - negative	Surgery performed - positive for recurrent disease	No follow-up	Other
Non-diagnostic	11	9	2	0	0	
Inflammatory	4	2	0	0	2	
Bland Follicular Cells	4	3	0	1	0	
Suspicious for Malignancy	4	1	0	2	0	1 - paraganglioma
Malignant	5	0	0	5	0	

Conclusions: Radiographically-guided FNA of the thyroid bed has a high non-diagnostic rate due to the occult and hypocellular nature of this post-therapeutic site. The proposed diagnostic categories are easy to follow and provide useful information for clinical management. While clinical follow-up and/or repeat FNA may be sufficient for the first three categories, the suspicious/malignant categories have a high positive predictive value and warrant further management.

365 Impact of Second Review of Thyroid FNA on the Implementation of Bethesda Classification: An Analysis of Cases on a Consult Service

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Background: The Bethesda system for reporting thyroid cytopathology (BSRTC) stratifies thyroid FNAs into 6 main diagnostic categories for clarity of communication among pathologists, surgeons and endocrinologists, and for appropriate triage of patients. Each of the categories has an implied cancer risk that ensures a rational clinical management guideline. This study was designed to determine the frequency of the use of BSRTC by referral laboratories and its implications on patient management.

Design: A retrospective search of our consult database revealed 693 cases with surgical follow-up during the period of January 2008 when we implemented the Bethesda system to December 2011. The cases were stratified based on the type of referral institution. The percentages of the original diagnoses based on the BSRTC were recorded.

Results: Referrals from community hospitals accounted for 80.8% of the 693 cases while private laboratories and academic institutions accounted for 15.6% and 3.6%, respectively. Over the 4-year study period, an average of 73.15% (SD 3.0%) of the original diagnoses were based on the BSRTC. Implementation rates for academic institutions, community hospitals and private laboratories were 72.0%, 73.4% and 71.3%, respectively. Of the 187 cases where the BSRTC was not implemented, 48 (25.7%) were reported without the use of any of the 6 primary diagnostic categories; 12 (13.8%) were reported using 2 diagnostic categories (overwhelmingly atypical/ suspicious categories). Seventy-one of these 187 cases (38%) turned out to be malignant on surgical resection. For those cases using the BSRTC, the original diagnoses were "atypia of undetermined significance" (AUS) in 6.1% of the cases. The rate of malignancy on subsequent surgical follow up of these AUS cases was 58.1%.

Conclusions: Five years after the guidelines were proposed, reporting of thyroid FNA still varies significantly from one laboratory to another, creating confusion in some cases and hindering the sharing of clinically meaningful data among laboratories. The BSRTC was not utilized in 1 out of 4 thyroid specimens. There was no statistically significant difference in the rate of utilization of BSRTC among different types of referral laboratories.

366 Cytopathologic and Molecular Diagnostic Clues to Poorly Differentiated Thyroid Carcinoma: A 10-Year Single Institution Experience

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Background: Poorly differentiated thyroid carcinoma (PDTC) is a rare thyroid neoplasm with clinical behavior between differentiated thyroid carcinoma and anaplastic thyroid carcinoma. PDTC often displays insular architecture, necrosis and increased mitotic activity on histopathologic evaluation. However, there are few studies describing cytological features of this rare tumor, which are important for a preoperative diagnosis. In this retrospective study, we review our experience in the fine needle aspiration (FNA) diagnosis of PDTC.

Design: Cases of PDTC were collected from our archives, a tertiary referral hospital, between the years 2002-2012. Of 21 cases surgically diagnosed as PDTC, 16 patients had preoperative FNA evaluation and were included in this study. Four cases had BRAF mutation analysis in addition to cytological evaluation. These cases were retrospectively reviewed by two cytopathologists.

Results: Eleven patients were men and five were women. The mean age was 56 years, ranging from 22 to 70 years. The original cytological diagnoses (16 patients) were as follows: 1 "benign" (FNA performed on one calcified nodule contralateral to eventual malignancy), 7 follicular neoplasm, 2 positive for malignancy-NOS, 4 papillary thyroid carcinoma (PTC) and 2 PDTC. Nine cytological specimens were available for review, the cytological features of which were listed in Table 1. Four cases were tested for BRAF mutation; all were negative.

Table 1. Cytomorphology of Poorly Differentiated Thyroid Carcinoma

Cytomorphological features	Case numbers (n)	Percentage (%)
High cellularity	9	100
Absence of colloid	7	77.8
Trabecular architecture	6	66.7
Numerous single cells	6	66.7
Abundant cytoplasm	6	66.7
Pleomorphism	4	44.4
papillary pattern	3	33.3
Microfollicular pattern	3	33.3
plasmacytoid cells	3	33.3
Vacuoles	1	11.1
Endothelial wrapping	1	11.1
Necrosis	0	0
Mitotic figures	0	0

Conclusions: Our results illustrate that PDTC can be difficult to diagnose on cytological specimens. However, in FNA samples with high cellularity, absence of colloid, a trabecular architectural pattern and numerous single cells that exhibit pleomorphism with abundant cytoplasm, a diagnosis of PDTC should be considered. Necrosis and mitotic figures are very helpful, but unfortunately, are very rare in cytological specimens. BRAF mutation testing does not assist with the diagnosis.

367 FNA Evaluation of Axillary Lymph Nodes for Breast Cancer Strongly Correlates with Surgical Pathology

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Background: Breast carcinoma is the most common malignancy and the leading cause of death for women in the United States. Axillary lymph node status is the most important prognostic factor. Pre-operative assessment of lymph node status by fine needle aspiration (FNA) is increasingly desired for assessment of lymph node status prior to neoadjuvant therapy. Moreover, surgical excision of axillary lymph nodes has sequela, including lymphedema, pain, shoulder restriction, numbness and weakness. However, there is limited literature assessing the correlation of FNA results with surgical excision.

Design: A search of the pathology database from 1/07 to 9/12 for cytology cases with keyword axilla was conducted. Two hundred and forty cases, where patients had a history of a breast mass or breast carcinoma and an axillary lymph node fine needle aspiration, were identified. One hundred thirty eight cases had follow-up core biopsy, sentinel node biopsy, or axillary dissection. Correlation was calculated using the Goodman-Kruskal Gamma. Cases with discordant results were reviewed by the authors to determine the cause of the discrepancy.

Results: Of the 138 cases, 132 (96%) were definitively positive or negative on cytology; 109 (83%) were concordant with the surgical result (Goodman-Kruskal Gamma = 0.84). Of the 23 discrepant cases, 5 were positive on cytology but negative on surgical excision; all of these patients had neoadjuvant therapy, and the cases are interpreted as true positives with interval complete tumor response. The other 18 cases were negative on cytology but positive on surgical excision; 2 were called isolated tumor cells or micrometastasis, and the rest had partial involvement of the lymph node by a small volume of tumor, and were classified as cytology sampling error. The 6 cases for which a definitive diagnosis could not be rendered were also due to insufficient cytology sampling: 3 were called atypical due to very rare abnormal cells, with 2 positive and 1 negative on surgical excision. Three cases were non-diagnostic on cytology due to lack of lymph node sampling. Overall, the positive predictive value of axillary FNA was 100% and the negative predictive value was 78%.

Conclusions: FNA cytology of axillary lymph nodes is strongly correlated with surgical pathology diagnosis, with 78% NPV, 100% PPV and a very low non-diagnostic rate. All discrepancies appear to be due to sampling error during FNA, mostly in lymph nodes with small tumor burden. The findings support the role of pre-treatment FNA evaluation of axillary lymph node status in patients with breast carcinoma.

368 Histoplasmosis: Have EUS and EBUS FNA Changed the Cytologic Face of the Disease?

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Background: Cytologic diagnosis of histoplasmosis is well-described and most series and reports focus on immunosuppressed patients with disseminated disease. However, with the advent of ultrasound-guided (US) fine needle aspiration (FNA) techniques, especially endoscopic (EUS) and endobronchial (EBUS) modalities, we have noted a marked increase in the cytologic diagnosis of histoplasmosis in immunocompetent patients.

Design: A computer search identified cytology cases with *Histoplasma* present in the past 10 years. All cases in which cytology material was available were included in this study, along with patient demographic, clinical and laboratory data.

Results: Forty cases of histoplasmosis (all FNA; 30 EBUS, 8 EUS, 2 US) were identified. The patients ranged from 15-86 years (mean 42); 22 female; 18 male; 36 of 40 (90%) patients were immunocompetent; only four were immunocompromised. Fourteen patients were being staged for primary tumors of other sites; others presented with primary pulmonary symptoms or were noted incidentally. Sites included lung and mediastinum (19), lymph nodes (18), adrenal gland (1), right neck (1), thyroid bed (1). The character of the specimens in all patients included: bland acellular necrosis with (31) or without (9) granulomas. In immunocompetent patients very rare intracellular organisms were seen on rapid or routine stains (Diff Quik and Pap) with variable (rare to many) extracellular organisms on GMS stain; a conspicuous neutrophilic infiltrate and giant cells were only occasionally seen (3 and 4 cases, respectively). In immunosuppressed patients the character of the necrosis was similar, but many more organisms were identified. Fungal cultures were negative in all patients except one (immunosuppressed; blood). *Histoplasma* yeast antigen (by compliment fixation, CF) was undetectable (<1:8) in 8/21 (sensitivity 61.9%)) and the mycelial antigen (CF) was undetectable in 20/21 (95.3%). *Histoplasma* urine antigen was detected in only 2/17 (11.8%).

Conclusions: 1. Disseminated histoplasmosis diagnosed cytologically has abundant intracellular organisms apparent on routine cytologic stains and confirmed on fungal stains.

2. Histoplasmosis in immune competent patients is characterized cytologically by bland necrosis with or without granulomas and requires fungal stains for diagnosis.

3. Urine and serum antigen tests have a low sensitivity (11.8% and 61.9%, respectively).

4. EUS and EBUS likely account for the increased cytologic diagnosis of histoplasmosis in immune competent patients, as nearly all of our cases were by these modalities.

369 Does Cytological Examination of Ovarian Cyst Fluids Obtained during Oophorectomy Add Useful Information?

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Background: Ovarian cyst fluid obtained during oophorectomy is frequently submitted for cytological examination. However, the value of cytologic examination of this sample is unclear, given that the ovarian lesion is undergoing excision and histologic examination. The aim of this study was to determine the accuracy of cytologic examination of the ovarian cyst fluid obtained during oophorectomy.

Design: A 10-year retrospective review was performed on ovarian cyst fluids obtained during oophorectomy. The fluid samples had been received fresh as a routine clinical sample and processed into a single ThinPrep slide. Cell blocks and ancillary studies were performed on selected cases at the discretion of the examining pathologist. Retrospectively, the cytology reports were reviewed and the cytologic diagnoses were categorized as benign, suspicious, unclassified neoplasm, malignant and non-diagnostic (cyst contents and insufficient cellularity). Histologic diagnoses on the excised ovarian lesion were categorized into benign, suspicious or malignant (including borderline tumors).

Results: 114 ovarian cyst fluids obtained during oophorectomy were identified from 112 patients. The mean patient age was 52 years (median: 49.5, range: 20 to 86 years). The mean cyst fluid volume was 49 mL (range 1 to 1000 mL). The results of the cytology and histology interpretations are presented in Table 1.

Table 1. Comparison of Cytology Classification of Ovarian Cyst Fluid and Histologic Classification.

	Histologic Classification of Ovarian Lesion			
Cytology Classification	**Benign**	**Malignant**	**Suspicious**	**Total**
Benign	25	7	0	32
Unclassified Neoplasm	8	8	0	16
Suspicious	2	8	1	11
Malignant	0	22	0	22
Non-Diagnostic	28	5	0	33
Total	63	50	1	114

Excluding the one suspicious histologic diagnosis and including all abnormal cytologic diagnosis (neoplasm, suspicious and malignant) as one category, the sensitivity, specificity, positive and negative predictive values, were 56.3%, 84.4%, 79.2% and 39.7%, respectively.

Conclusions: Malignant diagnoses from cytologic examination of the ovarian cyst fluid obtained during oophorectomy is highly accurate. However, the high rate of non-diagnostic samples, inaccuracies of benign diagnoses and large number of equivocal cytologic diagnoses render cytologic examination of cyst fluid obtained during oophorectomy of little value.

370 Follow-Up Outcomes of a Large Cohort of Low-Risk Women with ASC-US Imaged Liquid-Based Cytology and Negative HPV Test Results

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Background: New cervical screening guidelines propose a 5 year screening interval for the co-testing of women 30-65 years, and, for the first time, regard an ASC-US Pap test with negative high-risk (hr) HPV result (ANR) no different than a "double negative" (DNR) one. Long-term follow up data supporting this recommendation in the literature is very limited.

Design: We identified 6,852 patients 21 and older with ANR from computer-imaged ThinPrep and Hybrid Capture 2 hrHPV tests, screened between January 2006 and December 2006. 5 year follow-up data was collected from our laboratory information system including cytology, surgical, and HPV results. The follow-up diagnosis was assigned in decreasing order of significance: Cancer/CIN2-3/CIN1/Benign/HSIL/LSIL. Cytology diagnoses were selected only when no surgical pathology follow-up was available.

Results: Follow-up findings of CIN2/3/HSIL (HG) and CIN1/LSIL (LG) were detected in 1.3% and 7.8%, respectively. In women older than 30, HG was found in 26 of 4078 (0.55%), significantly lower than that in women 30 or younger (2.9%, 51/1766). No cases of invasive squamous cell carcinoma were identified. LG and HG results were detected in a significantly greater proportion of women with subsequent positive hrHPV results than in women with negative hrHPV results (60% vs. 5.4% $p<0.001$). The HG detection rate in women with ANR was significantly higher than that in women with DNR (0.3%, 12/4112, $p<0.0001$), but significantly lower than that in women with ASC-US and positive HPV testing (APR) results (5.1%, 111/2192, $p<0.0001$) in our institution.

5-year Follow-Up Findings after ANR Stratified by Age Group

	Total ANR	With Follow-Up	LSIL/CIN1	HSIL/CIN2/3
21-30 yr	2143	1766 (82.4%)	213 (12.1%)	51 (2.9%)
31-40 yr	1764	1497 (84.9%)	106 (7.1%)	13 (0.9%)
41-50 yr	1873	1639 (87.5%)	97 (5.9%)	8 (0.5%)
51-60 yr	838	747 (89.1%)	35 (4.7%)	3 (0.4%)
>60 yr	234	195 (83.3%)	3 (1.5%)	2 (1.0%)
Total	6852	5844	454	77

Conclusions: Our findings indicate that the risk of HG lesions in women with ANR is different from women with APR and women with DNR. That said, women older than 30 with ANR have a very low risk of CIN2+ lesions. This finding reaffirms the new recommendations by the United States Preventive Services Task Force and various professional societies.

371 Correlation of Tumor Cell Percentage and Absolute Cell Count with *EGFR* and *KRAS* Analyses Using Cytological Specimens. A Retrospective Study of 118 Cases

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Background: Lung adenocarcinoma is the leading cause of cancer deaths in the United States. *EGFR* and *KRAS* mutational analysis is critical to guide treatment with tyrosine-kinase inhibitors. Fine-needle aspiration cytology is an established method for diagnosing and staging lung cancer. In this study, we correlated the *EGFR/KRAS* mutational status in primary and metastatic non-small cell lung cancer with the percentage and absolute number of tumor cells using cytological specimens.

Design: Using pathology archives from our academic center, 118 cases of cytology lung adenocarcinomas submitted for molecular tests were identified from July 2008 to Sept 2011. Cell block sections were reviewed by pathologists to confirm diagnoses. Formalin fixed paraffin embedded sections were used for *EGFR* and *KRAS* analysis. Mutational status was determined by sequencing exons 18 to 21 of *EGFR* and codons 12 and 13 of *KRAS*. Retrospective counting of tumor cells was done on the cytology H&E slides, using visual estimation and a computer-aided automated segmentation algorithm.

Results: Of the 118 cases, 97 had sufficient tumor material for *EGFR* and *KRAS* analysis and H+E slides. Of the 97 cases, 22 cases (22.7%) were primary lung lesions. Metastatic sites included lymph nodes (51/75, 68%), pleura (12/75, 16%), and others (12/75, 16%). *EGFR* and *KRAS* mutations were detected in 23/97 (23.7%) cases and 35/97 cases (36.1%), respectively. The correlation of percentage and absolute cell number count is summarized in the **Table 1**. The minimum number of tumor cells in cytological samples that had a *KRAS* analysis was approximately 100 cells, whereas, *EGFR* tests were done in specimens containing approximately 200 or more tumor cells.

Conclusions: In this case series, positive *EGFR* test results were generally detected in specimens with greater numbers of tumor cells than for positive *KRAS* test results. In addition to the variable sensitivities of analytic methods used for studying *EGFR* and *KRAS* mutations, it is well-known that both DNA quantity and quality are critical for molecular analysis. Our study suggests that the absolute tumor cell count (likely indicative of DNA quantity) is an important criterion, in addition to tumor cell percentage. Further study is needed to examine the quality of DNA in cytological material and correlation with test results.

Percentage and Absolute Cell Count by Visual Estimation

% Tumor	N Samples	Mean Cell Count	Range	Standard Error of the Mean	Pearson
20-59%	20	290	100-1000	58.4	0.17
60-79%	24	442	100-1000	61.1	0.12
80-100%	53	1058	100-10,000	196	0.41

372 Utility of Axillary Lymph Node Fine-Needle-Aspiration in Management of Patients with Breast Cancer

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Background: Locoregional axillary lymph node status has long been the gold standard in determining the management and prognosis of breast cancer patients. Axillary lymph node fine needle aspiration (FNA) is an important confirmatory test for locally advanced and/or inoperable carcinoma. We present our institutional experience with axillary lymph node FNA in management of patients with breast cancer.

Design: The study included all patients with history of breast cancer that underwent axillary lymph node FNA over a 9 year period and had available subsequent follow up. Cytopathologic findings were reported as "positive/ suspicious for malignancy" and "negative". FNA results were correlated with surgical pathology outcome (considered the gold standard). The impact of axillary lymph node FNA on subsequent management decisions was also assessed.

Results: Of the 42 cases, axillary FNA was positive/suspicious for malignancy in 26 of 42 (62%) and, negative in 16 of 42 (38%). Palpation guided FNA and ultrasound guided FNA of axillary lymph nodes was performed in 22 (52%) and 20 (48%) cases, respectively. Surgical follow up was available in 41 of these cases. Sensitivity, specificity, positive predictive values and negative predictive values of axillary lymph node FNA were 83%, 100%, 100% and 69%, respectively. Four of 26 positive/suspicious FNA cases represented recurrences in the axilla and were surgically excised. Of the remaining 22 positive/suspicious FNA cases, 21 (96%) were spared sentinel lymph node biopsy and underwent axillary lymph node dissection. Of the 16 negative FNA cases, 10 (62.5%) underwent sentinel lymph node biopsy protocol and 6 underwent axillary lymph node dissection without sentinel lymph node biopsy.

Conclusions: Axillary lymph node FNA has high sensitivity and specificity for assessment of lymph node involvement by breast cancer and obviates the need for sentinel lymph node biopsy in patients with cytopathologic findings that are positive/ suspicious for malignancy.

373 Intraductal Tubulopapillary Neoplasm (ITPN) of Pancreas: Is Molecular Analysis of Cyst Fluid a Useful Adjunct in Distinguishing This Newly Recognized Tumor from Intraductal Papillary Mucinous Neoplasm (IPMN)?

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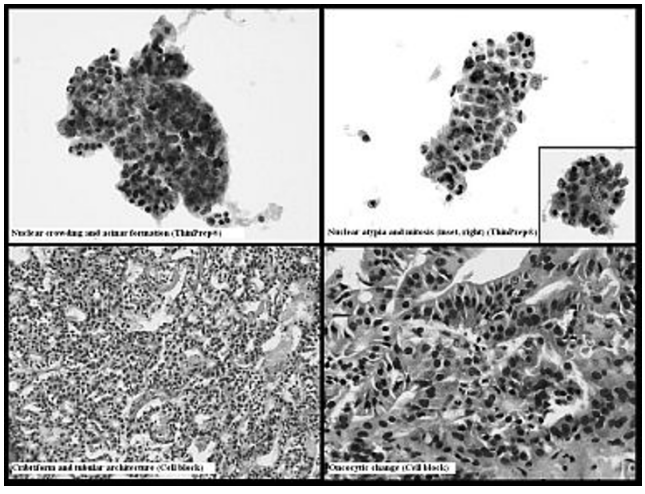
Background: ITPN was recently recognized as a distinct pancreatic tumor (WHO 2010). It is a rare indolent neoplasm that may be associated with dysplasia or invasive carcinoma. The main differential diagnosis of ITPN is IPMN as both tumors are intraductal and present with solid and cystic components. Histologically, ITPN is characterized by tubulopapillary and cribriform architecture and absent mucin. However, its cytomorphology and molecular aberrations have not yet been well described. This study presents the cytologic features of 2 cases of ITPN and compares the results of their molecular analysis with other pancreatic cystic lesions.

Design: Our database was searched from August 2010-April 2012 for endoscopic ultrasound-guided fine-needle aspirations (FNA) of pancreatic cystic lesions with available histologic follow-up and molecular analysis of the fluid [KRAS point mutations & tumor suppressor genes (LOH)]. Eight cases were identified, 2 of which histologically confirmed ITPN. The cytologic features of ITPN were evaluated and the molecular analyses, cytologic and histologic results were reviewed.

Results: Histologic diagnoses included 2 ITPN, 1 pseudocyst & 5 IPMN. Table 1 summarizes the cytohistologic correlation and results of fluid analysis. FNA of ITPN were cellular with a prominent tubular and cribriform architecture and absent mucin (Fig. 1). KRAS mutations were not detected in ITPN or pseudocyst, but were detected in all 5 IPMN, 4 of which had nondiagnostic FNA.

Surgical Dx	FNA Dx	DNA Quantity	KRAS	LOH
ITPN+insitu AC	Favor ITPN	+++	(-)	(-)
ITPN+AC	C/W ITPN	++	(-)	(-)
Pseudocyst	Cyst contents	++	(-)	(-)
IPMN-LG	ND	+++	(+)	(-)
IPMN-LG	ND	++	(+)	(-)
IPMN-LG	Mucinous lesion	++	(+)	(-)
IPMN+AC	ND	+++	(+)	(-)
IPMN+AC	ND	+++	(+)	(-)

ND=Nondiagnostic; AC= Invasive adenoca; LG=low grade dysplasia; ++= Moderate; +++= Highest;



Conclusions: The cytologic features of ITPN, namely lack of mucin coupled with tubulopapillary and cribriform architecture, are distinctive. In contrast to IPMN, KRAS mutations were not detected in ITPN. Our results suggest that cytomorphology along with molecular analysis can distinguish ITPN from IPMN.

374 Utility of Parathyroid Hormone Rinse Levels in Conjunction with Fine Needle Aspiration Cytology in the Evaluation of Indeterminate Parathyroid Neck Nodules

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Background: Fine needle aspirates (FNAs) of nodules radiographically indeterminate for parathyroid (PT) vs. thyroid tissue or unsuspected parathyroid lesions (PTL) can be diagnostically challenging. This study evaluates the utility of chemical analysis for parathyroid hormone performed on the aspirate rinse solution (PTH-r) in diagnosing these indeterminate lesions.

Design: The pathology database was searched for patients (pts) who had an ultrasound (US) guided FNA of the head and neck region with concurrent PTH-r analysis. The PTH-r levels were correlated with the cytologic features, histologic findings, and clinical data including serum calcium (Ca++) and intact serum PTH (PTH-s) levels. In this study, PTH-r was considered elevated if >1000 pg/dL.

Results: There were 10 pts (5 men and 5 women) who met the study criteria. By US, the FNA sites were designated as parathyroid (3 cases), thyroid (1 cases), indeterminate for PT (4 cases), thyroid bed (1 case), and neck (1 case). Nodules ranged in size from 0.8 to 3 cm (mean, 2.2 cm) in largest diameter. Elevated PTH-s and serum Ca++ levels were present in 7 of 8 and 5 of 9 pts tested, respectively. Six pts had elevated PTH-r levels, and in 5 of these, the cytologic diagnoses were PTL or PTL was in the differential; the other was insufficient for diagnosis (cystic lesion). Histologic follow-up was available for 6

pts. In 5 pts with high PTH-r levels, histology showed 4 PT adenomas (1 intrathyroid) and 1 PT hyperplasia. One pt without elevated PTH-r levels, but PTL on cytology, had a PT adenoma on excision. The remaining 4 pts were followed clinically, including 1 pt with high PTH-r levels and MEN1 syndrome with recurrent hyperparathyroid disease.
Conclusions: FNA with PTH-r has utility in differentiating PT from thyroid lesions in indeterminate nodules by US, FNA or paucicellular specimens. It is important to correlate the cytologic findings with the PTH-r levels. FNA with PTH-r is a useful approach in guiding clinical management, especially in patients with elevated serum Ca++ and/or PTH levels.

375 EGFR and KRas Mutation Analysis in Lung Adenocarcinoma: Adequacy of Cytology Samples

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Background: Availability of targeted therapies for lung adenocarcinoma (ADCA) with epidermal growth factor receptor gene (EGFR) or Kirsten rat sarcoma virus oncogene homolog (KRas) mutation compels early testing for tumor mutations. Although cytologic specimens are increasingly being used for molecular testing (MT), adequacy of these samples for evaluation of EGFR and KRas mutation is controversial. In this study, cytologic sample adequacy for MT is assessed in a lung ADCA series.
Design: Lung ADCA cytology specimens on which MT was performed were identified retrospectively. All MT was performed on cell block (CB) sections prepared using either CytoLyt™ or CytoRich™ Red. Total number of tumor cells ("cellularity") and percentage of nucleated cells that are tumor cells ("proportion") for each CB were independently evaluated by 3 cytologists and compared to MT results. EGFR and KRas were evaluated by PCR-based methods by a reference laboratory with tumor microdissection where necessary.
Results: Nineteen lung ADCA cytology samples were identified. Of these, EGFR was evaluated on 18, KRas on 4, and both on 3. A majority contained more than 400 tumor cells (11/19, 58%) and greater than 50% tumor proportion (13/19, 68%). Only samples containing fewer than 400 tumor cells were insufficient (QNS) for MT, and a majority of such samples had less than 50% tumor proportion. Rate of mutation detection was compatible with that in the general population (*Couraud. Eur J Cancer. 2012;48:1299*). CBs from all EGFR positive (+) samples contained high tumor cellularity and proportion; one CB from a KRas (+) sample contained low tumor cellularity and proportion.

Molecular Testing Results by Tumor Cellularity and Proportion (# of cases)

		EGFR			KRas	
		+	-	QNS	+	-
Tumor Cells (#)	**<50**	0	0	1	1	0
	50-400	0	3	4	0	1
	>400	3	7	0	0	2
Tumor Proportion (%)	**<50**	0	2	4	1	0
	>50	3	8	1	0	3

For 3 cases, a negative EGFR MT result was concordant between cytology CB and corresponding tissue section. One tissue section corresponding to a QNS cytology sample was EGFR (+).
Conclusions: This study demonstrates that 13/18 (72%) lung ADCA cytology samples had sufficient material for EGFR testing, and all 4 were sufficient for KRas testing. All EGFR (+) cytology samples were highly cellular, suggesting the possibility of false negative MT in samples of low tumor cellularity and proportion. However, KRas mutation was successfully detected in a sample of low tumor cellularity and proportion.

376 Urine Cytology in Pediatric Patients: A Clinicopathologic Correlation of 191 Cases

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Background: Urine cytology is an important diagnostic tool in the detection of bladder neoplasms and other pathologic processes of the genitourinary tract. Neoplasms of the urinary tract are distinctively uncommon in the pediatric age group, however non-neoplastic disease processes are fairly common. This present study evaluated the clinical utility of urine cytology exclusively in the pediatric population.
Design: A retrospective search was done for all urine samples examined in the cytology unit of our department from 1995 to 2010. A total of 191 urinary specimens (representing 171 patients) from patients 1-18 years of age were identified. The cytologic and follow-up clinical data was systematically analyzed and correlated.
Results: The median age was 9 years (range, 3 months-17 years) with a gender distribution of 112 male and 59 female (M:F=2:1). Most common presenting symptom was hematuria, followed by frequency. The largest diagnostic group consisted of benign/normal cytopathologic findings (88%) or displayed acute inflammation (21%), reactive changes (24%), and hematuria (40%). Crystals were identified in 2% of cases and polyoma virus features were present in 1.6%. One case was suspicious for malignancy while 21 cases were called atypical based largely on architectural atypia. A total of 11 cases had surgical follow-up. The great majority of these (55%) had urinary calculi. One benign neoplastic case (leiomyoma) was identified, while 2 cases progressed to end stage renal disease. Thirteen cases with atypia were not followed by either repeat urines or surgical biopsies. No urothelial neoplasm was identified by urinary cytology in the study group.
Conclusions: A large majority of urinary specimens in pediatric age group are benign, display reactive changes or hematuria. Urothelial neoplasms are a rarity and were not identified in any of the cases in our study group. Cytologic atypia in pediatric age group is an uncommon category, and not associated with cancer follow-up. Majority of cases with atypical diagnosis in pediatric age group are benign, with no need for follow-up repeat urines or tissue biopsies.

377 Diagnostic Error Assessment of EUS-FNA of Neuroendocrine Neoplasms of the Pancreas

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Background: Standardization of error classification in anatomic pathology has become an important issue due to the difficulty in accurate meta-analysis of error rates and causes. Zarbo et al (Arch Pathol Lab Med – Vol 129, Oct 2005) have proposed taxonomy and measurement tools to obviate previous inconsistency in error reporting. The objective of this study is to assess the extent of error occurring in cytopathologic diagnosis of neuroendocrine lesions of the pancreas, classify these errors and determine their impact on clinical outcomes.
Design: We collected information on all cases diagnosed as neuroendocrine neoplasm either by EUS-FNA in cytology or by surgical pathology from 2000-2012. Utilizing the standardized error and harm classification noted above, we reviewed the cytology and surgical pathology material and evaluated the type and the cause of diagnostic errors and their impact on the patient.
Results: During the study period, 177 patients who had EUS-FNA were diagnosed with a neuroendocrine neoplasm either by cytology or by surgical pathology (age range 15-90 yrs, 91 males). 80 of these cases had surgical follow up at our institution with surgical specimens available for review. Of these 80 cases, 56 had an adequate cell block from the EUS-FNA and immunohistochemistry was performed. There were 13 discrepancies between cytologic and surgical pathologic diagnoses (16% of EUS-FNA cases with surgical follow up). There were no false positive cases, 9 false negative cases (69% of total errors, 14% of cases with follow up) and 4 misclassifications (31% of total errors, 5% of cases with follow up). The false negative cases consisted of 3 interpretation errors and 6 cytology sampling errors. The misclassifications consisted of 3 cases of solid pseudopapillary neoplasm diagnosed as neuroendocrine neoplasm on cytology and one case of neuroendocrine carcinoma diagnosed as adenocarcinoma on cytology. There were no surgical pathology errors. All errors were associated with no or minimal harm, as the false negative cases received surgical intervention due to high clinical or radiologic suspicion. There was no moderate or major harm.
Conclusions: This study demonstrates error rates and misclassification associated with neuroendocrine neoplasms of the pancreas. EUS-FNA of pancreatic neuroendocrine neoplasms has an excellent positive predictive value, with no false positive diagnoses made in this 12-year study. When an adequate sample is obtained, the most significant error is misclassification, most often associated with solid pseudopapillary neoplasm. The impact of these errors is minimal.

378 Telepathology Assisted Immediate Assessment of EBUS-FNA Has Equivalent Accuracy to Cytopathologist On-Site Examination

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Background: Performing immediate assessment (IA) has become the standard of care for Endobronchial Ultrasound-Guided Fine Needle Aspiration (EBUS-FNA) specimens. Despite the benefits of aiding interventional pulmonologists to achieve higher adequacy rates and fewer unnecessary passes, the time required by attending cytopathologists to be present for on-site assessments is significant and impacts other clinical responsibilities. At our institution, it is a brisk 9 minute walk from the cytology department to the EBUS-FNA suite. Telepathology, as implemented here, consists of a cytotechnologist or trainee driven microscope attached to a Nikon DS-Fi1 Camera and DS-L2 controller that displays dynamic microscopic images in real-time on the attending pathologist's office computer.
Design: Preliminary assessment results, final diagnoses, and corresponding surgical pathology diagnoses, when available, were compared between consecutive EBUS-FNA specimens acquired before and after implementation of telepathology assisted IA. Cases were divided into three categories: satisfactory for evaluation (SFI), indeterminate for evaluation (IND), and unsatisfactory for evaluation (UNSAT).
Results: 105 EBUS-FNA specimens that were evaluated by conventional on-site examination were compared to the first 116 cases using telepathology assisted IA. The IAs for the conventional on-site cases were 60/105 (57%) SFI; 34/105 (32%) IND; 11 (10%) UNSAT. The telepathology assisted cases were 58/116 (50%) SFI; 47/116 (40%) IND; 11/116 (10%) UNSAT. A 3x2 Fisher's exact test showed no significant difference in adequacy assessment distribution between the two methods (p=0.45). Conventional on-site IA and telepathology assisted IA had similar abilities to predict which samples would be diagnostic on final cytologic examination. 59/60 (98%) and 57/58 (98%) of SAT cases (p=1), 29/34 (85%) and 44/47 (94%) of IND cases (p=0.27) and 4/11 (36%) and 8/11 (73%) of UNSAT cases were diagnostic on final examination, for conventional on-site exam and telepathology assisted, respectively. 26 of the conventional on-site cases and 25 telepathology assisted on-site cases had corresponding tissue diagnoses with a single discrepant result in each group.
Conclusions: Telepathology assisted IA of EBUS-FNA allowed for the same diagnostic accuracy as traditional on-site IA with the added advantage of eliminating travel time for attending pathologists.

379 MYB Translocation t(6;9) in Adenoid Cystic Carcinoma Fine Needle Aspiration Biopsy Using Fluorescence In-Situ Hybridization

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Background: Salivary gland neoplasms evaluated by fine needle aspiration (FNA) biopsy can have a wide variety of appearances with considerable morphologic overlap between various benign and malignant entities. A general morphologic categorization of "basaloid" neoplasm is a frequently encountered pattern and diagnostic challenge. The main differential diagnostic considerations include benign mixed tumors/monomorphic

adenoma (BMT) and adenoid cystic carcinoma (ACC). There is consensus agreement that FNA biopsy cytomorphology alone does not permit distinction. The value of distinguishing the two entities is related to their biologic behavior and initial surgical approach. Chromosomal abnormalities of ACCs have not been commonly evaluated in FNA biopsy material. MYB gene translocation t(6;9) (q22-23;p23-24) has been identified in around 50% of formalin fixed paraffin embedded (FFPE) tissue and has been absent in other salivary gland neoplasms, including benign mixed tumors.
Design: Patients who underwent FNA biopsy for known and surgically confirmed ACC and BMT were identified and smears from air-dried modified Wright-Giemsa stained slides selected. The direct smear slide was used for fluorescence in-situ hybridization (FISH) utilizing a protocol optimized for direct FNA smears. Commercially available red and green fluorescent labeled probes, hybridizing to MYB-telomeric and MYB-centromeric, were used to identify the MYB gene and evaluate for a translocation. Using a fluorescent microscope, at least fifty DAPI stained non-overlapping cells of interest were counted per case.
Results: 6 ACCs and 5 BMT FNA cases underwent FISH evaluation for MYB translocation. Utilizing a fluorescent microscope and DAPI stained nuclei; at least fifty cells of interest were evaluated for the presence of the MYB gene and evidence of a break-apart signal. One half of the ACCs (3/6) showed a positive break-apart signal in the majority of the 50 cells counted. Of the 5 BMTs, none of the 50 cells counted showed a MYB translocation.
Conclusions: The MYB gene was present by FISH evaluation in the BMT and ACC FNA smears. The BMT cases demonstrated the MYB gene without evidence of translocation. For the ACC cases, the MYB translocation was present in half the cases (50%). This corresponds to the reported prevalence in FFPE tissue for ACC surgical resections. Utilizing FISH testing of salivary gland FNA cases for MYB translocation t(6:9) in select cases has the potential to provide additional specific diagnostic information.

380 "Atypia of Undetermined Significance/Follicular Lesion of Undetermined Significance" in Bethesda System for Reporting Thyroid Cytopathology: Prediction of Malignant Risk in Subcategories with BRAF Mutation Results

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Background: The "Atypia of Undetermined Significance/Follicular Lesion of Undetermined Significance (AUS/FLUS)" category in the Bethesda System for Reporting Thyroid Cytopathology is a heterogeneous category of cases that are not clearly benign or malignant.
Design: We conducted a retrospective analyses correlating cytologic and histologic evaluation of thyroid nodules which were interpreted as 'AUS/FLUS' category on initial fine needle aspiration (FNA) in our institution for 13 months. The cases in AUS/FLUS category were classified into two subgroups according to the predominance of nuclear atypia (AUS) or microfollicular architecture (FLUS). Additionally, in a proportion of those cases, analyses for BRAF gene mutations were performed.
Results: Of 6402 thyroid FNAs, 551 (8.6%) were diagnosed as AUS/FLUS. Of the 551 cases, 431 (6.7% of 6402) were AUS and 120 (1.9% of 6402) were FLUS. Follow up cytologic or histologic outcome data were available for 388 cases which consisted of 315 AUS and 73 FLUS. Of 388 cases, repeated FNA without histologic evaluation were performed in 157 cases and they were diagnosed as follows: unsatisfactory, 1.9%; benign, 59.9%; AUS/FLUS, 21.6%; suspicious for a follicular neoplasm, 2.5%; suspicious for malignancy, 5.1% and malignancy, 8.9%. Histologic confirmation with or without repeated FNA data were obtained in 231 cases, of which 74 (32%) were benign and 157 (68%) were malignant. In the AUS, 21.2% (41/193) were benign and 78.8% (152/193) were malignant on histologic outcome. In contrast, in the FLUS, 86.8% (33/38) were benign and 13.2% (5/38) were malignant on histologic outcome (P<.001). Meanwhile, in the AUS/FLUS, 135 cases had adequate BRAF mutation analysis results and were accompanied with histologic diagnosis. BRAF mutations were found in 87 AUS cases, 86 of which were papillary carcinoma. In contrast, there was no BRAF mutation in FLUS. In correlating the molecular results with histologic outcome, we found that the cancer probability for AUS cases with BRAF mutation was 98.9%, while the cancer probability for those cases without BRAF mutation was 68.8% (P<.001).
Conclusions: The subcategory 'AUS' indicates a higher risk of malignancy than subcategory 'FLUS'. Therefore, it is important to report the subcategorized group of AUS/FLUS with potential clinical implication. In addition, BRAF molecular test is helpful in predicting malignant risk of AUS cases.

381 Endoscopic Ultrasound-Guided Fine-Needle Aspiration of the Pancreas: A Retrospective Study of 1,000 Cases

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Background: Although endoscopic ultrasound (EUS)-guided fine-needle aspiration (FNA) is an established method for the diagnosis of pancreatic tumors, only a few large studies have appeared in the literature.
Design: A computerized search of our pathology LIS was performed and all EUS-guided FNAs of the pancreas performed for the 7-year period from 2004 through 2011 were identified. Each cytologic diagnosis was classified as cystic or solid and placed into one of 6 categories: benign (B), atypical (A), suspicious (S), malignant (M), tumor (T) and non-diagnostic (ND). A complementary search for all related surgical pathology reports was also performed and stratified in the same manner. For the purpose of determining the degree of correlation, all non-diagnostic and atypical diagnoses were excluded from consideration and suspicious, malignant, and tumor diagnoses were categorized as positive.
Results: A total of 1,000 EUS-guided FNAs of the pancreas were identified. Of the cases, 579 were solid lesions obtained from 301 female and 278 male patients with an age range of 19-95 years (mean 63). The FNA diagnoses of the solid lesions were: B 229 (39.5%), A 22 (3.8%), S 27 (4.7%), M 260 (44.9%), T 1 (0.2%), and ND 40 (6.9%). The malignant FNA diagnoses included 209 adenocarcinomas, 21 metastases, 10 pancreatic neuroendocrine tumor (PNET), 6 solid pseudopapillary tumors, 2 lymphomas, 1 malignant giant cell tumor, 1 anaplastic carcinoma and 1 squamous cell carcinoma. Of the 579 FNAs of solid pancreatic masses, 145 had corresponding surgical follow-up. The sensitivity and specificity for solid lesions were 91.5% and 100% respectively. There were 9 false negative cases due to sampling error and specimen hypocellularity. There were 421 cystic lesions from 254 female and 167 male patients with an age range of 20-96 years (mean 63). The FNAs of the cystic lesions were classified as follows: B 343 (81.5%), A 5 (1.2%), S 5 (1.2%), M 7 (1.7%), T 44 (10.5%), and ND 17 (4.0%). Of the 421 cystic FNA cases, 96 cases had corresponding surgical follow-up. There were 40 cystic neoplasms failed to be diagnosed by FNA including 1 adenocarcinoma, 2 PNETs, 6 MCN, 8 serous cystadenomas and 23 IPMN. The sensitivity and specificity to identify tumor for cystic lesions were 34.4% and 93.3% respectively.
Conclusions: At our institution, EUS-guided FNA of solid pancreatic masses is both sensitive and specific for the diagnosis of both primary and metastatic tumors. For cystic lesions, FNA is not sensitive in identifying low-grade IPMN, MCN or serous cystadenoma, but its specificity remains high.

382 Utility of Thyroglobluin Rinse Levels as an Adjuct to FNA of Neck Nodules in the Evaluation of Metastatic Papillary Thyroid Carcinoma

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Background: Ultrasound (US) is often used to evaluate patients (pts) for metastatic or recurrent papillary thyroid carcinoma (PTC) of the neck. US-guided fine needle aspiration (FNA) of abnormal nodules is often diagnostic of disease; however, in some instances lesions are small or cystic yielding insufficient number of cells for diagnosis. This study evaluates the utility of measuring thyroglobulin in the aspirate rinse (TG-r) as an adjunct to cytology in evaluating PTC pts for metastatic or recurrent disease.
Design: The pathology database was searched for pts with concurrent US-guided FNAs and TG-r levels performed from 2008-2012. There were 51 FNAs obtained from 45 pts (26 women, 19 men; age range: 20-79 years) meeting the study criteria. Of the 45 pts, 33 had a history of PTC, while the remaining had none. FNA sites included 41 non-thyroidal (25 cervical nodes, 3 supraclavicular nodes, 11 soft tissue, and 2 parotid glands) and 10 thyroidal (9 thyroid bed and 1 thyroid). Based on an ROC curve, 8ng/ml was chosen as a cutoff value for Tg-r (area under curve: .893 {95% CI, .780-1.000}). TG-r levels were correlated with the concurrent cytology diagnoses, subsequent histology, and clinical data.
Results: Of the 41 nonthyroidal FNAs, 27 had elevated TG-r levels, and the concurrent cytology diagnoses were positive for PTC in 7, suspicious for PTC in 6, atypical in 3, negative for PTC in 11. Of these, 20 showed metastatic PTC on subsequent excision or FNA, while 7 were followed clinically. Of the 10 thyroidal FNAs, 8 had elevated TG-r levels and the cytology diagnoses were suspicious for PTC in 1, atypical in 4, negative for PTC in 1 and nondiagnostic in 2. Four of these showed metastatic PTC on subsequent excision and 4 were followed clinically. There were 16 FNAs with nonelevated TG-r levels of which 4 showed PTC on excision, 4 showed no evidence of PTC, and the remainder were followed clinically. The mean TG-r level for cases with PTC on excision was 9365, versus 3 ng/ml for pts with no evidence of PTC on excision. The sensitivity and specificity for TG-r in all sites were 86% and 100%, whereas sensitivity and specificity of a positive/suspicious cytology was 63% and 100%, respectively.
Conclusions: TG-r levels are a useful adjunct to cytology in evaluating radiographically abnormal lymph nodes for metastatic PTC in selective cases, especially if cystic by US or paucicellular on FNA. Our data suggests that while a negative TG-r level does not entirely rule out PTC, markedly elevated levels in aspirates should prompt further investigation for recurrent PTC.

383 Histologic Follow-Up for Atypical Endocervical Cells

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Background: In previous literature on atypical glandular cells, using conventional and non-imaged liquid-based Papanicoalou (Pap) tests, squamous dysplasia was about twice as likely to be found on follow-up as glandular neoplasia (Davey et al. APLM 2000). We have examined the performance of atypical endocervical cell categories in our experience with imager-aided liquid-based screening.
Design: Cases from the categories of atypical endocervical cells (AECC), atypical endocervical cells favor neoplastic (FN), and atypical endocervical cells favor adenocarcinoma in situ (AIS) were collected. These were correlated with subsequent histologic follow-up. Cases were stratified into two patient groups: a dysplasia clinic and the general population. The two-tailed Fischer exact test was used to calculate p-values.
Results: Over a six year period, 120 cases were identified with tissue follow-up available in 76 (63.3%).

	AECC	AIS	FN	Total
	N=86	N=15	N=19	N=120
Available tissue follow-up	49 (56.9%)	10 (66.7%)	17 (89.5%)	76 (63.3%)
Average age (range)	40 (19-66)	36 (28-58)	34 (21-67)	37.5 (19-67)
HGSIL (CIN 2/3)	8†	5‡	6†	19 (25.0%)
LGSIL (HPV/CIN I)	7*,**	0	2	9 (11.8%)
AIS	5*,†	4‡	4†	13 (16.1%)
Cervical adenocarcinoma	1	1	0	2 (2.6%)
Endometrial adenocarcinoma	1**	0	1	2 (2.6%)
Total	19	7	12	38 (50%)
*1 with AIS and LGSIL				
**1 with enodometrial adenocarcinoma and LGSIL				
†1 with AIS and HGSIL				
‡3 with AIS and HGSIL				

Dysplasia clinic patients were more likely to have interpretations of FN and AIS versus AECC (17/34) when compared to other patients (17/86) and were also more likely to have positive findings on histologic follow-up (20/34 versus 17/86, p=0.0089). When looking at all patients, AIS and FN were more likely to show significant findings on histologic follow up than AECC (p-value=0.016). However, AIS and FN had similar follow-up positive rates (p-value=1.0).

Conclusions: Relative to prior studies, we had a much higher proportion of the follow-up biopsies that showed glandular neoplasia. Our data comes from a context of imager-assisted liquid-based screening. One explanation is that this technology has helped us to more accurately identify squamous lesions, accounting for our relatively high yield. These categories performed better in a high-risk population than in the general population. AIS and FN performed similarly to each other, and much better than AECC.

384 Assessment of Diagnostic Yield of Traditional 22G FNA and Core Biopsy with the New 22G Echotip Procure Needle in Sampling of Solid Pancreatic Masses

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Background: EUS-guided FNA is widely utilized for sampling and diagnosis of pancreatic lesions. The Echotip Procore Needle (EPN) (Wilson-Cook Medical) is a new 22G biopsy needle with reverse bevel designed to obtain core biopsy material with retrograde motion at time of EUS. The aim of this study was to compare the diagnostic yields of conventional EUS FNA and EPN.

Design: All pancreatic lesions included in this study were solid (>60%) pancreatic masses diagnosed on imaging and confirmed at time of EUS. All pancreatic lesions were sampled by both FNA and core biopsy. The patients were randomized to receive FNA (22G Echotip Ultra, Wilson-Cook Medical) or core biopsy (22G EPN, Wilson-Cook Medical) by first attempt. An on-site evaluation of all FNA material was performed. FNA material was processed per routine cytologic methods. Core biopsy material was formalin fixed, processed, paraffin embedded, and subsequently stained with hematoxylin and eosin. FNA and core biopsy material were assessed for accuracy of diagnosis, cellularity (0-3), presence of contamination (0-3), and sufficiency for ancillary studies. Diagnostic performances were compared.

Results: 32 patients (13 men, 19 women, mean age = 68 yrs) met the study criteria. 23 lesions were located in the head, 6 in the body, and 3 in the tail of the pancreas. FNA was performed first (before EPN) in 16 patients. FNA material was superior to core biopsy in reaching a diagnosis in 22 cases and was identical to EPN in diagnostic utility in 8 cases. Neither FNA nor EPN arrived at the correct diagnosis in 1 case. EPN was superior to FNA in only 1 patient with metastatic renal cell carcinoma to the pancreas. Further comparisons are summarized in Table 1.

FNA to EPN Comparison

	FNA	EPN
Technical success (%)	100	84
Diagnostic yield (%)	94	33
Cellularity (mean)	2.9	1.63
Contamination (mean)	0.81	0.42
Sufficiency for ancillary studies (%)	78	67

Conclusions: In spite of functioning as a core biopsy needle, this study demonstrates that the technical success and diagnostic yield with the new 22G Echotip Procore needle is not comparable to traditional 22G FNA of solid pancreatic lesions sampled with EUS. Further studies may be helpful to establish the diagnostic utility of the EPN.

385 Comparative Study for Diagnosis of Gastrointestinal Submucosal Lesions (GISML) Using Standard EUS-Fine Needle Aspiration (FNA) Versus EUS-Fine Needle Biopsy (FNB)

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Background: GISML are difficult to diagnose with EUS-FNA (sensitivity: 61-91%; specificity: 61-100%). The mesenchymal nature of most of these lesions makes it difficult to obtain reliable samples for cytological diagnosis with the standard FNA needle. A new EUS-guided biopsy needle with side fenestration (ProCore) was developed to enable EUS-FNB. Our aim was to compare results using the standard FNA 19G needle with those using the new FNB 19G device (ProCore).

Design: 20 consecutive patients who underwent EUS for evaluation of GISML were enrolled in a pilot study (June 2011-February 2012), using the standard 19G FNA needle and the new 19G FNB ProCore device. Previous radiological diagnoses were: 9 GISTs, 6 leiomyomas, 3 lipomas and 2 heterotopic pancreas. For every case, FNA and FNB with on-site-evaluation (OSE) were performed (1-3 passes each). For all cases, FNA smears and touch preps of FNB were obtained; cell blocks (CB) from FNA were available in 13/20 and tissue core (TC) from FNB in 20/20. IHC was performed when appropriate.

Results: Size of GISML was 25 ± 14 mm (mean ± SD). 5 were located in the oesophagus, 12 in gastric camera, 3 in duodenum. Cytological diagnosis with FNA was possible for 6/20 patients, and for 4/20 with FNB. Histological diagnosis was possible for 6/13 CB (FNA) and for 9/20 TC (FNB). Combining cytology and histology from both devices, a total of 10/20 patients were diagnosed: 6 GISTs, 3 leiomyomas, and 1 neuroendocrine tumor (NET), confirmed by IHC stains and surgical biopsy. Remaining cases (10/20) were reported "unsatisfactory" with both needles. FNA was diagnostic for 8/20 patients and FNB for 9/20. Both devices were coincident in diagnosis for 7/20 patients. Of the remaining 3, 2 were diagnosed as leiomyoma by FNB and 1 as NET by FNA. Previous radiological diagnoses by EUS were confirmed in 9/10 cases (6 GISTs, 3 leiomyomas); NET was diagnosed as GIST by previous EUS.

Conclusions: Although diagnosis remains difficult in GISML, mostly of mesenchymal origin, FNB seems to have a slight advantage over FNA. With the same caliber, FNB provides superior tissue samples. Our results show an overall diagnostic yield of 50% for the combined technique of EUS-FNA-FNB with specificity of 100%. 7/10 lesions that remain undiagnosed had a size between 10-18 mm that could explain the low sensitivity in this series. In our experience, improvement in results can be achieved through choosing the appropriate device, and combining cytology, histology and OSE.

386 Claudin-4 Immunohistochemistry Is Highly Effective in Distinguishing Adenocarcinoma from Malignant Mesothelioma in Effusion Cytology

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Background: Adenocarcinoma (ACA) can be challenging to distinguish from malignant mesothelioma in effusions, and often requires ancillary studies and clinical data. Immunohistochemical panels, including calretinin, WT-1, EMA, AE1/AE3, MOC31, BerEP4, and B72.3, are useful but can yield varying results. Immunohistochemistry for claudin-4, a tight-junction associated protein, has recently been shown to distinguish ACA from malignant mesothelioma, mostly in surgical specimens. Our aim is to validate and assess immunoreactivity for claudin-4 staining in a large series of malignant effusions.

Design: 148 malignant effusions (84 ACA, 64 malignant mesothelioma) diagnosed between 2008-2012 were retrieved from the cytopathology files. Medical records and pathology reports were reviewed for each case. For all cases of ACA, either a known primary or confirmatory IHC were required for inclusion. For all malignant mesotheliomas, concurrent confirmatory studies were required (biopsy/resection, cytogenetics, or immunohistochemical studies). Immunohistochemistry was performed on cell block sections following heat-induced epitope retrieval (EDTA/steamer) using a monoclonal antibody to claudin-4 (clone 3E2C1) and an immunoperoxidase technique. Moderate-to-strong membranous staining in greater than 50% of tumor cells was considered a "positive" result. Absent or weak/focal cytoplasmic staining was considered "negative."

Results: 64 cases of mesothelioma and 84 ACA (31 lung, 2 esophageal, 2 gastric, 4 colon, 1 appendix, 3 pancreas, 15 ovarian, 24 breast, 1 prostate, 1 unknown primary) were evaluated. 83 cases of ACA were positive for claudin-4 (99%); 1 case of serous carcinoma was negative. Most cases of ACA showed strong and diffuse membranous staining (71/84; 84%); 12 (14%) cases showed membranous staining of moderate intensity. All cases of mesothelioma were negative for claudin-4.

Conclusions: Claudin-4 immunohistochemistry effectively distinguishes ACA from malignant mesothelioma with high sensitivity and specificity. Addition of claudin-4 to the typical immunohistochemical panel when encountered with a challenging malignant effusion has high utility. In addition, the characteristic strong staining pattern may be useful in detecting malignant ACA cells in samples with scant cellularity.

387 Lubricant-Related High Unsatisfactory Rate with ThinPrep – Can the Cellularity Be Improved?

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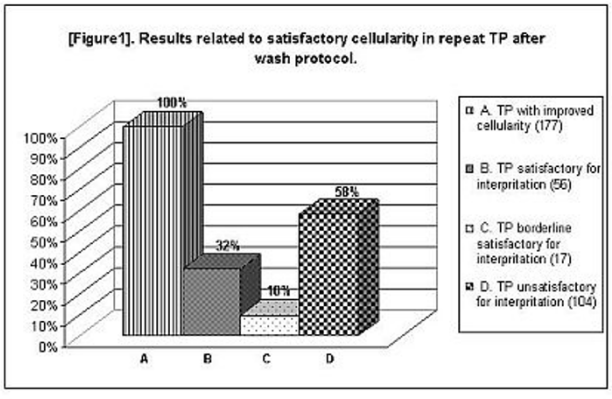
Background: As compared to other cervical cytology specimens, ThinPrep® (TP) has relatively higher unsatisfactory rate (3.5%). Interference due to *lubricant-like debris* (LUBE) is one of the most common causes for unsatisfactory rate for TP. A prospective study was performed after IRB approval to address this limitation.

Design: 1000 cases of unsatisfactory TP (over 5½ months) were analyzed for different causes. A standardized protocol based on the principle of washing with PreservCyt® was applied to prepare repeat TP if the residual specimen was available. All repeat TP were evaluated by 2 cytotechnologists followed by 6 cytopathologists.

Results: Out of 1000 specimens, 677 were unsatisfactory due to scant cellularity with LUBE interference. Other causes such as scant cellularity, blood, atrophic cellular changes, inflammation, atrophic vaginitis etc. were observed in remaining cases. 33/1000 (3%) specimens were virtually acellular. Out of 530 available residual specimens, 377 (71%) with LUBE were analyzed after repeat TP. Improved cellularity was noted in 177 (47%) specimens, but only 73 (42%) were satisfactory. 56 (32%) cases were cellular and 17 (10%) cases showed borderline cellularity (just over 5,000 cells per TP) (**Figure 1**).



Satisfactory repeat TP showed 89% NILM, 8% ASCUS, 1% LSIL, 1% ASC-H, 1% AGUS. These numbers were compared with the routine results on satisfactory TP in same population during similar period, studied as six different slots of sample sizes

comparable to the current numbers over one year period which overlapped with this study. Statistical analysis with *Chi-square test* showed statistically insignificant *p value* more than 0.05 confirming that the results related to detection of abnormal cells in repeat TP were comparable to the general trend.

Conclusions: 1. A repeat TP with a simple wash protocol is recommended to improve unsatisfactory rate of TP cervical cytology without affecting the final interpretation.
2. Although the cellularity improved in 47% of specimens, only 19% (73 out of 377) showed adequately diagnostic cellularity. This emphasizes the significance of avoiding lubricant contamination as the preferred recommendation.

388 Targeted Next-Generation Sequencing from Fine Needle Aspirates

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Background: Molecular testing of cancer is increasingly critical to medical care. Next-generation sequencing (NGS) provides comprehensive, unbiased, and inexpensive mutation analysis of multiple target genes with a single test. However, the utility of NGS on fine needle aspirate (FNA) material, which is often the only specimen available, is unknown. Non-small cell lung cancer (NSCLC) is an ideal model in which to evaluate the application of NGS to cytopathology since FNA is frequently used for diagnosis and staging, and specific molecular therapeutic targets have been identified in NSCLC. We evaluated the performance and quality of targeted NGS in FNAs from a small series of lung adenocarcinomas.

Design: Sequence data were generated from FNAs (percutaneous CT-guided or endobronchial) and paired formalin-fixed paraffin-embedded (FFPE) tissue resections from 5 patients with lung adenocarcinoma. DNA was isolated from cells scraped from both Diff-Quik (DQ) and Papanicolaou (Pap) stained FNA slides. Indexed sequencing libraries were prepared from specimens with ≥ 100 ng DNA. Multiplex, paired-end sequencing of 27 cancer-related genes was performed after hybrid capture enrichment. A custom pipeline based on the Genome Analysis Toolkit was used to identify unique genomic variants from mapped reads. Read quality metrics and single nucleotide variant (SNV) calls were compared across sample types.

Results: Average concordance of single nucleotide variants across specimen types was >99.99%. A small difference (p=0.028) in the percentage of total mapped reads between DQ (98.45%) and FFPE (99.03%) specimens was observed; however, the percentages of on target and duplicate reads did not differ statistically (p> 0.05) between FFPE and cytologic preparations. Between the DQ and Pap preparations there were no differences in mapped reads, on target reads, duplicate reads, or sequence variant calls.

Conclusions: DNA derived from routine FNA fixatives performs comparably to FFPE derived tissue in NGS assays. DNA isolated from the two main types of FNA specimens, DQ and Pap stained slides, yields comprehensive and accurate sequence information, which is statistically indistinguishable from that obtained from FFPE tissue. These results demonstrate the utility of FNAs to provide extensive, high-quality molecular characterization of tumors and support the integration of NGS technologies into the standard cytopathology workflow.

389 A Feasibility Study Comparing Single Pathologist Review, Consensus Review, and FISH for Diagnosis in Endometrial Brush Cytology

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Background: Endometrial Brush Cytology (EBC) has been proposed as a less invasive alternative to endometrial biopsy in women being evaluated for abnormal uterine bleeding, but the method has not gained popularity due to perceived challenges in interpretation and accuracy. Fluorescence in situ hybridization (FISH) has been tried as an adjunct test to improve diagnostic performance, but implementation is cost-prohibitive. The aim of this study was to evaluate various approaches to EBC diagnosis to improve accuracy.

Design: Fifty EBC specimens had been collected immediately prior to hysterectomy and were processed using the ThinPrep method. These included 30 with benign and 20 with cancer (16 endometrioid, 4 non-endometrioid) histology. Seven blinded pathologists reviewed pre-screened slides after a 1 hour training session discussing diagnostic criteria with example cases. Pathologists classified the cases as non-diagnostic (ND), negative (N), atypical favor reactive (AFR), atypical favor neoplastic (AFN), or positive for malignancy (P). These screening levels were collapsed into negative (ND, N, AFR) and positive (AFN, P) for the purpose of statistical analysis. All cases were evaluated by FISH using probes directed to 1q25, 8p11, 8q24, and 20q13. Single locus gains and polysomy were considered positive, while normal results, tetrasomy, and ND were considered negative. Performance characteristics were calculated for FISH, the worst case scenario (WCS) single pathologist, random single pathologist, and consensus of 3 random panel pathologists (for each case), as well as combinations thereof.

Results: Table 1.

Performance Characteristics of EBC

	FISH	WCS	Single	Consensus	WCS + FISH	Single + FISH	Consensus + FISH
Sensitivity	0.70	0.70	0.95	1.00	0.90	0.95	1.00
Specificity	0.87	0.47	0.83	0.90	0.43	0.73	0.80
PPV	0.78	0.47	0.79	0.87	0.51	0.70	0.77
NPV	0.81	0.70	0.96	1.00	0.87	0.96	1.00

WCS = worst case scenario pathologist; Single = single random pathologist; Consensus = consensus of 3 random pathologists

Conclusions: Three pathologist consensus outperformed a random single pathologist. The addition of FISH improved the performance of the WCS pathologist, but did not improve the performance of a randomly selected pathologist from the panel. These results suggest that traditional single cytopathologist approaches might not be optimal for EBC interpretation, and that consensus approaches should be considered to improve accuracy. These findings are preliminary and need to be validated on a larger prospective cohort with population disease prevalence.

390 Renal Cell Carcinoma with Rhabdoid Features: Cytologic Features on Fine Needle Aspiration Biopsy

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Background: Rhabdoid differentiation (RD) in renal cell carcinoma (RCC) is associated with poor prognosis. Distinction of RD from oncocytic renal neoplasms is challenging due to overlapping cytologic features. Fine needle aspiration (FNA) cytology of RCC with RD is not well-described. Our aim was to establish consistent cytologic features to detect RD in FNA biopsies.

Design: We performed a search for cases of primary or metastatic RCC with RD with concurrent FNA. Cases were reviewed for RD and compared to consecutive FNA of oncocytic kidney neoplasms with histologic follow up. Cytologic parameters were defined (Table 1), and cases were screened by a cytotechnologist and a fellow. Fisher exact test was performed to determine if each parameter of RD was significantly different when compared to conventional oncocytic neoplasms.

Results: 76 specimens contained RCC with RD: 68 clear cell RCC (CCRCC), 1 papillary RCC, 1 chromophobe RCC (ChRCC) and 6 unclassified RCC. 6 of these had FNA; 2 cases were from primary kidney tumors and 4 from metastases (2 lung, 1 liver, 1 distant lymph node). RCC with RD were compared to 17 conventional (non-RD) oncocytic tumors (6 CCRCC, 3ChRCC, 4 oncocytomas, and 4 benign kidneys with fibrosis). 6 parameters on FNA were significantly associated with RCC with RD (p<.01): irregular nuclear membrane, vesicular nuclei, eccentric nuclei, vesicular cytoplasm, intracytoplasmic inclusions and ill-defined cell borders (Table 1).

Conclusions: In this series, we describe key cytologic features of RCC with RD, which were even identified in metastases. As RD is associated with poor prognosis, the presence of characteristic parameters on initial FNA may signify a more aggressive neoplasm suggesting early definitive management.

Cytologic parameters in RCC with rhabdoid features

	Non rhabdoid (n=17)		Rhabdoid (n=6)		
Follow up histology (n=23)	Parameter absent	Parameter present	Parameter absent	Parameter present	p-value (Fisher exact test)
Nuclear enlargement	2 (12%)	15 (88%)	0	6 (100%)	0.53
Irregular nuclear membrane	17 (100%)	0	0	6 (100%)	<.001
Vesicular nuclei	15 (88%)	2 (12%)	0	6 (100%)	<.001
Central nuclei	2 (12%)	15 (88%)	6 (100%)	0	<.001
Eccentric nuclei	15 (88%)	2 (12%)	0	6 (100%)	.003
Prominent nucleolus	8 (47%)	9 (53%)	5 (83%)	1 (17%)	.3401
Granular cytoplasm	17 (100%)	0	6 (100%)	0	<.001
Vesicular cytoplasm	15 (88%)	2 (12%)	0	6 (100%)	.0003
Intracytoplasmic inclusion	17 (100%)	0	3 (50%)	3 (50%)	.0113
Well-defined cell borders	7 (41%)	10 (59%)	6 (100%)	0	.0191
Ill-defined cell borders	11 (65%)	6 (35%)	0	6 (100%)	.0137
Cohesive sheets	6 (35%)	11 (65%)	6 (100%)	0	.0137
Loss of cohesion	3 (18%)	14 (82%)	0	6 (100%)	.5392

391 The Impact of Using the Bethesda System for Reporting Thyroid Cytology Diagnostic Criteria for the AUS/FLUS Category

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Background: AUS/FLUS has been a problematic diagnostic category due to it's predictive uncertainty for neoplasm or malignancy. To address terminology and other issues related to AUS/FLUS interpretation, the Bethesda System for Reporting Thyroid Cytology (BSRTC) refines definition and provides specific diagnostic criteria for the AUS/FLUS category. Our institution has implemented BSRTC since January 2011. The goal of this study was to review our institutional experience in regard to follow-up of thyroid nodules interpreted as AUS/FLUS using BSRTC diagnostic criteria.

Design: A SNOMED search of the electronic pathology in our institution for the period of 01/2011 to 06/2012 was conducted to retrieve thyroid aspirates previously interpreted as AUS/FLUS using the BSRTC diagnostic criteria. Information including specimen preparation method, names of the individual reviewers who initially assessed the specimens, availability of repeat fine needle aspiration and/or surgical intervention along with the corresponding cytologic and/or histologic diagnosis was collected. Cytology-histology concordance was evaluated for aspirates with surgical follow-up.

Results: 1. A total of 120 aspirates were retrieved. The aspirates were prepared as conventional smears (n=111) or ThinPrep (n=9).
2. A repeat fine needle aspiration was performed in 12.5% (15/120) of the AUS/FLUS cases. Among which, 4 cases remained as AUS/FLUS, 9 and 2 cases were re-categorized as benign and suspicious for neoplasm, respectively.
3. Surgical follow-up was available in 42.5% (51/120) of AUS/FLUS cases. Surgical interventions were performed following the initial and repeat AUS/FLUS interpretation in 94.1% (48/51) and 5.9% (3/51) of the surgically managed cases, respectively.
4. The follow-up histology revealed 27.5% (14/51) conventional papillary thyroid carcinoma, 23.5% (12/51) follicular adenoma and 49% (25/51) non-neoplastic nodules (nodular hyperplasia or lymphocytic thyroiditis).
5. The aspirates were originally evaluated by 8 individual cytopathologists with experience ranging from junior to senior level. The presence of neoplasia/malignancy in follow-up surgical specimens does not correlate with the level of the individual reviewer's experience.

Conclusions: In comparison to our previously reported data showing adenoma in 14.9% and carcinoma in 9.2% of the surgically managed AUS/FLUS cases, the current study demonstrates that adhering to the BSRTC diagnostic criteria yields a more reliable prediction of histology-proven follicular adenoma (23.5%) and malignancy (27.5%) for the AUS/FLUS category.

392 The Significance of Urothelial Cell Clusters in Voided Urine Specimens

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Background: The cytomorphologic examination of voided urine is commonly employed to screen for and detect urothelial cell carcinoma (UCC). The significance of the "atypical" interpretive category for urine cytology is uncertain. Adding to this uncertainty is the debatable significance of urothelial cell clusters in voided urine. The etiologic differential diagnosis includes urinary tract stones, recent urinary tract instrumentation, and neoplasia; however, some experts argue that urothelial cell clusters in voided urine should be regarded as benign findings. As the literature on the significance of urothelial cell clusters in voided urine is sparse, we retrospectively analyzed a large cohort of patients for whom clusters of urothelial cells were observed in voided urine specimens.

Design: A total of 542 patients, found to have clusters of urothelial cells in voided urine samples between 2000 and 2011, with at least one-year of clinical follow-up were identified. For each, the electronic medical records were examined for the following: prior history of UCC, cystoscopy notes, operative reports, radiology reports for urinary tract calculi, and subsequent histopathologic followup. For comparison of malignancy rates within this cohort, patients were stratified based on presence/absence of urinary tract calculi, prior history of UCC, and history of recent urinary tract instrumentation.

Results: Overall, UCC was diagnosed on follow-up surgical pathology specimens in 102 (18.8%) of 542 patients. Urinary tract calculi were present in 103 (19.0%) and absent in 439 (81.0%) of the 542 patients at the time of voided urine collection. UCC was subsequently diagnosed in 4 (3.9%) of 103 patients with calculi and in 98 (22.3%) of 439 patients without calculi ($p<0.0001$). Next, 171 (31.5%) and 371 (68.5%) of the 542 patients had or did not have a prior history of UCC at the time of voided urine collection, respectively. On follow-up, UCC was diagnosed in 57 (33.3%) of 171 patients with a prior history of UCC and 45 (12.1%) of 371 patients without a previous history of UCC ($p<0.0001$). Finally, a history of recent urinary tract instrumentation was documented in 17 (3.1%) of 542 patients; the malignancy rate in this subset of patients was 4 (23.5%) of 17. UCC was diagnosed on follow-up for 98 (18.7%) of 525 patients without a history of recent instrumentation ($p=0.54$).

Conclusions: The presence of urothelial cell clusters in voided urine is clinically relevant and should not be equated with a negative interpretation. A prior history of UCC and absence of urinary tract calculi represent significant predictors of UCC on follow-up.

393 Comparing Two Platforms of Detection for High Risk Human Papillomavirus in Both SurePath and ThinPrep Gynecologic Specimens

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Background: HR HPV DNA testing is currently recommended for the triage of all women with ASC-US and in conjunction with Pap tests for women >30. Most of the commercially available methods including the Hybrid Capture 2 (HC2) assay (Qiagen, Gaithersburg, MD) and the more recent Cobas 4800 (C4800) test (Roche, Branchburg, NJ) are approved by the FDA for ThinPrep (TP) (Hologic, Boxborough, MA), but not for SurePath (SP) (BD, Burlington, NC). As part of a validation process, we compared the performance of C4800 test and the HC2 assay in the detection of *HR HPV* using both TP and SP.

Design: A total of 1,371 liquid based gynecologic preparations, 1,122 SP and 249 TP specimens, were tested for *HR HPV DNA* using the C4800 and the HC2 assay. For discrepant test results between C4800 and HC2 assay, confirmatory test was performed using Linear Array HPV testing (Roche). The concordance between the CTQ and HC2 assays was analyzed using the kappa statistic.We calculated the diagnostic accuracy of both C4800 and HC2 assays. A sample was treated as a true negative if results of both the C4800 and the HC2 assay were negative and a true positive if results of both the C4800 and the HC2 assay were positive or if the result of the Linear Array test confirmed a positive assay result for cases with discordant results.

Results: Table 1 summarized the results of HR HPV DNA testing using both assays. The kappa value was 0.73. There was no statistical significant difference in the percentage of positive HPV results among SP and TP. A total of 82 (6.0%) cases demonstrate discordant HPV results, 70 (6.2%) cases were SP and 12 (4.8%) cases TP. ($p > 0.05$) 27 out of 82 (32.9%) cases were C4800-/HC2+ and 55 out of 82 (67.1%) were C4800+/HC2-; 10% C4800-/HC2+ and 70% C4800+/HC2- cases were confirmed positive for HR HPV by Linear Array.

Results of HPV DNA testing

Type of specimens	Number of cases tested negative for both assays	No. of cases tested positive for both assays	No. of discordant results between the 2 assays	Kappa Value	Diagnostic accuracy for C4800 assay	Diagnostic accuracy for HC II assay
SurePath (n=1,122)	949 (84.6%)	103 (9.2%)	70 (6.2%)	0.71	99.0%	95.2%
ThinPrep (n=249)	211 (84.7%)	26 (10.4%)	12 (4.8%)	0.79	99.6%	95.6%
Total (n=1,371)	1,160 (84.6%)	82 (6.0%)	82 (6.0%)	0.72	99.1%	95.3%

No. = number

Conclusions: Both assays showed a good agreement. The number of discordant results was relatively small. However, more false positive results were encountered with HC2 than C4800. The C4800 assay was found to be more accurate than HC2 assay in detecting HR HPV DNA in both SP and TP.

394 Is There a Role for High Risk HPV Testing in the Triage of Patients with ASC-H?

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Background: Current guidelines recommend high risk (hr) HPV DNA testing for patients with atypical squamous cells, not otherwise specified (ASC-US) but immediate colposcopy for patients with ASC, cannot rule out HSIL (ASC-H). The objective of this study is to investigate the role of hrHPV testing for colposcopy triage of ASC-H in routine practice, as there are no guidelines for its use in this setting.

Design: All liquid based Pap tests with the diagnosis of ASC-H were identified between Jan 2008- December 2011. hrHPV DNA testing either as cotesting or reflex testing was performed using hybrid capture II (Qiagen, Gaithersburg, MD). Patient demographics and results of histologic follow up were recorded.

Results: During the study period, a total of 630 Pap tests were diagnosed with ASC-H, accounting for 0.2% of all Pap tests evaluated during the study period. ASC-H accounted for 0.17% of all SurePath and 0.33% of all ThinPrep specimens; the difference was statistically significant. High risk HPV status was available in 586 (93%) cases; 396 (67.6%) were tested for hrHPV. Table 1 and 2 summarize the results of follow up according to hrHPV status, specimen type and two different age groups (< 30 vs ≥ 30). There were no statistically significant differences in the percentage of patients found to have a CIN2+ lesion with a negative hrHPV status between the two types of liquid based preparations or between the two different age groups.

Table 1.

hrHPV Negative Cases				
Follow-up SP Diagnosis	**Negative/CIN 1 (%)**	**CIN 2+ (%)**	**No FU (%)**	**Total**
SurePath	59.54	8.40	32.06	131
ThinPrep	77.97	5.08	16.95	59
<30 years	60.42	8.33	31.25	85
≥30 years	90.48	7.62	1.90	105
Total	65.26	7.37	27.37	190 (of 586)

Table 2.

hrHPV Positive Cases				
Follow-up SP Diagnosis	**Negative/CIN 1 (%)**	**CIN 2+ (%)**	**No FU (%)**	**Total**
SurePath	47.08	37.01	15.91	308
ThinPrep	47.73	38.64	13.64	88
< 30 years	37.84	41.08	21.08	185
≥ 30 years	62.23	37.77	0.00	211
Total	47.22	37.37	15.40	396 (of 586)

Conclusions: The incidence of ASC-H diagnoses was low for both liquid based preparations but the incidence of ASC-H of ThinPrep specimens was significantly higher than that of SurePath. hrHPV was positive in about 2/3 of the patients with ASC-H. 8% or less of patients with ASC-H and a negative hrHPV status were found to have a CIN2+ lesion. High risk HPV DNA testing is useful in triaging patients with a diagnosis of ASC-H regardless of the preparation type or age group.

395 Utility of Thyroglobulin Measurements in Fine Needle Aspiration Cytology of Lymph Nodes for the Detection of Metastatic Papillary Thyroid Carcinoma

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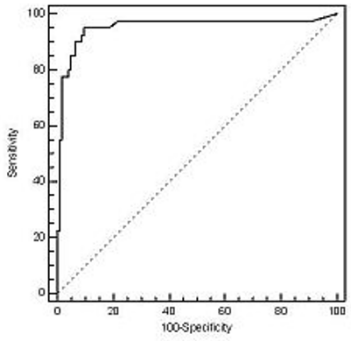
Background: Ultrasound-guided fine-needle aspiration (US-FNA) cytology is a commonly used method in the surveillance of suspicious lymph nodes (LN) in papillary thyroid carcinoma (PTC). The measurement of thyroglobulin (Tg) levels in LNs during FNA has been suggested to improve the diagnostic yield. We investigated the utility of US-FNA-Tg in the diagnosis of LN suspicious for metastatic PTC.

Design: 227 cases with both US-guided FNA cytology and US-FNA-Tg measurements from our academic center were included. Tg levels were correlated with cytological diagnosis and follow-up surgical resection specimen.

Results: In 41 cases of cytologically diagnosed metastatic PTC, 40 cases were confirmed surgically. The median US-FNA-Tg concentration was 4901.6 ng/ml; whereas, in 124 benign LNs the median Tg concentration was <0.2 ng/ml ($P<0.0001$). ROC analysis (AUC=0.962 demonstrated a sensitivity of 98% and specificity of 78% at the Tg detection limit (<0.2 ng/mL) (Figure 1), while cutoffs of 9.6 to 100 ng/mL resulted in a sensitivity of 78% and specificity of 98%. Of 15 cases with a cytological diagnosis of "suspicious for PTC", 9 cases had markedly elevated FNA-Tg. Seven of the 9 cases had follow-up surgeries confirming the diagnosis of PTC. Of 30 cases with a cytological diagnosis of "non-diagnostic", 8 cases had markedly elevated FNA-Tg with a median of 5,943 ng/ml. Each of 8 cases was confirmed to be metastatic PTC by surgical follow-up.

Figure 1. ROC analysis of US-FNA-Tg concentrations in lymph nodes with cytological diagnosis of benign (n=124) or cytological and surgical diagnosis of metastatic papillary thyroid cancer (n=40), AUC=0.962.



Conclusions: US-FNA-Tg demonstrates a strong negative predictive value (93%-99%). It may be particularly useful for difficult cases. However, standardization of the sample collection is still needed to further improve the accuracy of the approach.

396 Endoscopic Ultrasound-Guided Fine-Needle Aspiration (EUS FNA) of Cystic Pancreatic Lesions: What Is the Value of Molecular and Chemical Analysis of Cyst Fluid in Diagnostic and Nondiagnostic Samples?

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Background: Cystic lesions of the pancreas are often complex and heterogeneous with low diagnostic yield by EUS FNA. We evaluated whether molecular analysis for loss of heterozygosity (LOH) and KRAS point mutations, as well as chemical analysis for CEA, assisted in classifying these lesions.

Design: We identified 65 cystic pancreatic lesions collected by EUS FNA since 2010 that had cyst fluid sent for molecular testing and chemical analysis. The cytologic, histologic, molecular and chemical results were reviewed.

Results: Of 65 cases, EUS FNA results were nondiagnostic in 74% (48) compared with our overall nondiagnostic rate for pancreatic EUS FNA of 26%. The 48 nondiagnostic samples had material sent for molecular analysis. KRAS mutations were detected in 15 (31%) and 7 had surgical follow-up with results summarized in Table 1.

Table 1. Nondiagnostic EUS FNAs

Surgical follow-up	KRAS	LOH	CEA (ng/ml)*
IPMN, LG	Y	N	L
IPMN, LG	Y	N	H
AdenoCA	Y	N	L
AdenoCA	Y	N	H
MCN	N	N	H
MCN	N	N	H
MM	N	N	H

*L=low (<192) H=high (>192)

KRAS mutations were detected for intraductal papillary mucinous neoplasm with low grade dysplasia (IPMN, LG) and invasive adenocarcinoma (AdenoCA) but not in mucinous cystic neoplasms (MCN) or mucinous metaplasia (MM). Fluid analysis for CEA showed elevation in most mucinous lesions (except 1 IPMN, LG and 1 AdenoCA). Of 17 diagnostic EUS FNAs, 6 had surgical follow-up and are summarized in Table 2.

Table 2. Diagnostic EUS FNAs

FNA Diagnosis	Surgical follow-up	KRAS	LOH	CEA (ng/ml)*
Mucinous cystic lesion	IPMN, LG	Y	N	L
Mucinous cystic lesion	IPMN, inv CA	-	-	H
SPN	SPN	N	N	L
ITPN	ITPN	N	N	L
GIST	GIST	N	Y	L
PEN	PEN	N	Y	L

KRAS mutation was detected in IPMN, LG while the IPMN with invasive carcinoma (IPMN, inv CA) was not tested for KRAS but had elevated CEA. LOH was detected in a gastrointestinal stromal tumor (GIST) and a pancreatic endocrine tumor (PEN).

Conclusions: Our results show that a combination of KRAS mutations and/or high CEA levels identified all 8 mucinous neoplasms and AdenoCA (6 of which were nondiagnostic by EUS FNA). The remaining tumors (SPN, ITPN, GIST and PEN) had low CEA levels and no KRAS mutations. LOH was detected only in GIST and PEN. We conclude that when cytologic material is diagnostic, molecular and chemical analysis may not provide additional information. However, in nondiagnostic cases, cyst fluid analysis may be useful in identifying lesions that need close clinical follow-up.

397 Detection of *KRAS* and *BRAF* Hotspot Mutations on Cytology Smears in Metastatic Colorectal Carcinomas Using Pyrosequencing

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Background: Anti-EGFR therapy is hindered when *KRAS* or *BRAF* mutations are present. Currently, *KRAS/BRAF* testing is conducted on formalin-fixed paraffin-embedded (FFPE) tissue from primary colorectal cancer (CRC) which may not be readily available. In this study, we examined *KRAS/BRAF* mutations utilizing fine needle aspiration (FNA) smears of metastatic CRC as a more cost-effective, less invasive approach and investigated the *KRAS/BRAF* results between primary CRC and subsequent metastases.

Design: We received institutional funding to investigate 15 patients with CRC diagnosed on colectomy specimens and with subsequent metastases diagnosed on FNA cytology. All cases were reviewed by cyto- and surgical pathologists. Genomic DNA from marked areas was extracted and purified from 5-10 um FFPE sections and was extracted with and without purification from air-dried FNA smears. Qiagen® pyrosequencing assays were used to detect *KRAS* codons 12, 13, and 61 and *BRAF* codon 600 mutations.

Results: All primary specimens (100%) were successfully sequenced at all codons. *KRAS* mutations were detected in 6 of 15 (40%) primaries, and *BRAF* mutation in 2 of 15 (13%) cases. Sequencing was possible for all metastases (100%) but was dependent on extent of purification. Twelve of 15 (80%) FNA smears were successful using both crude and purified DNA. Two smear cases, 300 (*BRAF* only) and 500 cells (*KRAS/BRAF*), failed with crude DNA but were successful with purified DNA. The third case (400 cells) failed all assays with purified DNA but was successful with crude DNA. Discordance was demonstrated in one metastatic case (200 cells) which had wild-type *KRAS*, whereas the primary tumor had mutations in codons 13 (GGT>GAT) and 61 (CAA>CAT) at 26% and 21% mutant allele frequencies, respectively. No additional mutations were detected in the metastatic specimens.

Conclusions: Our *KRAS* and *BRAF* mutation rates in primary CRC specimens and our concordance rate were similar to the most recent literature. Of particular interest is the case that detected both codons 12 and 61 *KRAS* mutations in the primary tumor but showed wild-type results in FNA metastatic tumor cells. Importantly, when DNA was extracted by both crude extraction and further purification, *KRAS* and *BRAF* hotspot codons were successfully genotyped with few amplification inhibitors in all of our FNA specimens, supporting the use of FNA smear preparations when limitations of using primary CRC specimens exist.

398 Dedifferentiated Liposarcoma and Pleomorphic Liposarcoma: A Comparative Study on FNA Cytomorphology and MDM2/CDK4 Expression

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Background: Both dedifferentiated liposarcoma (DDLPS) and pleomorphic liposarcoma (PLPS) are high-grade liposarcomas. DDLPS is a non-lipogenic sarcoma evolving from a well-differentiated liposarcoma and is characterized by amplification of MDM2 and CDK4. PLPS is a high-grade sarcoma containing lipoblasts, characterized by a complex karyotype and a more aggressive clinical course. Rarely, DDLPS shows lipogenic differentiation, mimicking PLPS. Cytomorphologic features of DDLPS and PLPS are poorly characterized.

Design: Cytologic preparations of 24 DDLPS and 14 PLPS, histologically confirmed, were retrospectively reviewed. Cytomorphologic findings were correlated with clinical, histologic and genetic data. Immunohistochemistry for MDM2 and CDK4 was performed on cell blocks and/or core needle biopsies.

Results: FNA smears from both DDLPS and PLPS were variably cellular, composed mostly of loose cellular clusters, some with abundant myxoid stroma and/or branching curvilinear vessels. Tumors were predominantly composed of spindled (21 cases), epithelioid (11), or pleomorphic (6) atypical cells, with delicate fibrillary cytoplasm and variably shaped nuclei. Additional non-distinctive features included necrosis (11), mitoses (6), or a prominent inflammatory infiltrate (8). The main cytologic difference was the presence of abundant lipoblasts and cells with microvacuolated cytoplasm in most PLPS. Multinucleated giant cells and pleomorphic cells with bizarre nuclei were present in both tumor types, but more commonly in PLPS. Extracellular hyaline eosinophilic globules were present in 2 PLPS. Expression of MDM2 and CDK4 was detected in 23 (96%) and 20 (83%) DDLPS, but in none of the PLPS except for 1 case showing weak CDK4 reactivity. Clinical features and cytogenetic data also varied between DDLPS and PLPS. All these features are summarized in Table 1.

Table 1: Clinical, cytologic, immunohistochemical, and genetic features of DDLPS and PLPS.

		DDLPS (n=24)	PLPS (n=14)
Clinical	Median Age [range]	66.5 [43-81]	56 [22-80]
	Sex	15M:9F	10M:2F
	Site	Retroperitoneum (12), thigh (3), liver (3), intraabdominal (2), lung (2), others (2)	Extremities (8), mediastinum (2), pancreas (2), liver (1), intraabdominal (1)
	Type of lesion	Primary (3), recurrence (10), metastasis (6)	Primary (7), recurrence (1), metastasis (5)
Cytologic Features	Lipoblasts	2 (8%)	10 (71%)
	Microvacuolated cytoplasm	10 (42%)	13 (93%)
	Cells with bizarre nuclei	13 (54%)	10 (71%)
	Multinucleated Giant Cells	Occasional, 4 (17%)	Abundant, 6 (43%)
IHC	MDM2+	23 (96%)	0
	CDK4+	20 (83%)	1 (7%)
Genetics	Karyotype	Ring or giant marker chromosomes (4)	Complex (2)
	FISH	MDM2 amp (4)	-

Conclusions: DDLPS and PLPS exhibit variable and often overlapping cytologic features. The presence of lipoblasts and cells with microvacuolated cytoplasm is more suggestive of PLPS, but can occur in DDLPS. Coexpression of MDM2 and CDK4 distinguishes DDLPS from PLPS.

399 Human Papilloma Virus Testing and p16 Immunohistochemistry as Ancillary/Reflex Tests in ASC-H Cervical-Vaginal Cytology

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Background: 'Atypical squamous cells – cannot exclude HSIL' (ASC-H) category in The 2001 Bethesda Terminology System continues to be a challenging area. The current ASCCP *(American Society of Colposcopy and Cervical Pathology)* guidelines recommend a colposcopic biopsy evaluation for ASC-H. As a part of IRB approved study at our tertiary care institution with extensive outreach component during the period of Jan 2010 to Aug 2011, we analyzed ASC-H interpretations in ThinPrep cervical cytology (TP) in relation to surgical pathology findings and results of Digene Hybrid Capture 2 High-Risk HPV DNA Test (HPVT).

Design: The database was searched for availability of surgical pathology (including p16 immunostaining) and HPVT for all ASC-H cases during this period. Positive predictive value (PPV) and negative predictive value (NPV) were calculated for HPVT & p16 with reference to surgical pathology results in ASC-H cases.

Results: We studied 754 TP with ASC-H from 722 patients with a mean age of 35.3 years. HPVT was available in 300 cases (115 with biopsy & 185 without biopsy). The results of surgical pathology including p16 and HPVT were analyzed (Table 1).

Table 1. Surgical biopsy, p16 IHC, and HPVT characteristics of cases with ASC-H

Total cases: 132446 (Over 20 months - Jan 2010 to Aug 2011) in TP						
ASC-H Cases: 754						
Biopsy (276 cases)	HPVT with biopsy (115)	p16 IHC with HPVT (28)				p16 IHC without HPVT (35)
	P/N	p16 - P; HPV - P	p16 - P; HPV - N	p16 - N; HPV - P	p16 - N; HPV - N	P/N
No Dysplasia - 142 cases (51%)	11/50	0	0	1	6	0/12
CIN-1 - 58 cases (21%)	13/14	3	5	1	1	10/0
≥CIN-2 - 76 cases* (28%)	23/4	11	4	0	0	11/2*

CIN-1 includes both dysplasia and HPV changes. * Includes one case of endometrioid adenocarcinoma. P, positive; N, negative

Conclusions: 1. Although negative HPVT in ASC-H cases has a high NPV of 94% for HD in biopsy, an occasional case had false negative HPVT with positive HD in biopsy.
2. Comparable to ALTS recommendation for ASCUS, reflex HPVT is recommended as an adjunct to establish ASCCP type algorithm for managing the ASC-H cases.
3. p16 has a very high PPV of 100% for dysplasia in biopsies on ASC-H cases.
4. Although not evaluated directly during this study, based on the biopsy findings in this study and other reports related to the evaluation of p16 in liquid based cytology (both TP & SurePath), reflex application of p16 immunostaining on TP from residual sample is suggested.

400 Fine-Needle Aspiration of Hurthle Cell Lesions: A 10-Year Retrospective Study of 147 Cases

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Background: Hurthle cell change is not an uncommon finding in thyroid fine-needle aspiration (FNA) cytology, and can be associated with various non-neoplastic conditions as well as follicular neoplasms and thyroid carcinoma. Diagnostic challenges arise when attempting to classify Hurthle cell change as metaplastic or as suggestive or diagnostic of a Hurthle cell neoplasm.

Design: A computerized search of our laboratory information system was performed for the 10-year period from August 2002 through August 2012 to identify all thyroid FNA and correlating surgical pathology diagnoses that included Hurthle cell or oncocytic nomenclature. Those cases that did not have corresponding thyroidectomies after FNA were excluded. Based on the follow-up diagnosis, the risk of malignancy was calculated, after excluding cases of papillary microcarcinoma (PMC). The risk of neoplasm was calculated, including all malignancies, PMC and adenomas.

Results: A total of 147 FNA, thyroidectomy specimens and clinical histories were analyzed over a 10-year period. The patient ages ranged from 16 to 83 years, with a mean of 52. The female to male ratio was 3.9:1. The size of the lesions aspirated ranged from 0.2 cm to 9.0 cm. The FNA diagnoses were classified as follows: benign (12 cases, 8%), atypia of undetermined significance, cannot exclude Hurthle cell neoplasm (AUS-HCN) (56 cases, 38%), Hurthle cell neoplasm (HCN) (72 cases, 49%), suspicious for malignancy (5 cases, 3%), malignant (1 cases) and nondiagnostic (1 case). Of the 12 cases diagnosed as benign by FNA, 5 were adenomas, 5 were benign non-neoplastic (BNN), and 2 were follicular carcinomas. Of the HCN cases, follow-ups showed 31 adenomas, 15 BNN, 21 malignant and 5 PMC. Of the AUS-HCN cases, histology demonstrated 19 adenomas, 22 BNN, 7 malignant and 8 PMC. Those 5 FNAs classified as suspicious for malignancy yielded 1 adenoma, 1 BNN and 3 malignancies. The one malignant FNA was an oncocytic variant of papillary carcinoma. An adenoma was identified on histologic follow-up of the one nondiagnostic FNA.

Conclusions: The risks of malignancy and of neoplasm were 13% and 61% for AUS-HCN and 29% and 79 % for HCN, respectively. The risk of malignancy is within the 5-15% suggested for AUS and 15-30% suggested for follicular neoplasm, as described in the Bethesda System for Reporting Thyroid Cytopathology. One of the false negative cases was due to interpretation error and, in retrospect, should have been diagnosed as "AUS-HCN". The other false-negative case was an outside consultation and the cytologic smears were not available for review.

401 Characterization of the Atypical Diagnostic Category in Endoscopic Ultrasound-Guided Fine Needle Aspiration (EUS-FNA) of the Pancreas: A Ten Year Experience

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Background: Endoscopic ultrasound-guided fine needle aspiration (EUS-FNA) is a critical diagnostic and staging tool for solid and cystic lesions of the pancreas. Few studies have characterized the spectrum of lesions that fall into the atypical category and compared this result to concurrent core needle biopsy (CNB) and subsequent resection. We believe this is one of the first large studies of this kind.

Design: Data were collected retrospectively on all patients undergoing EUS-FNA at our institution from April 2002 to July 2012. Cases diagnosed by cytology as atypical were selected. The results of CNB and surgical resection specimens were obtained when available. Clinical data were collected from patient charts.

Results: A total of 1,106 EUS-FNA cases were identified from 2002 to 2012, of which 50 cases (4.5%) were categorized as atypical. Of the atypical cases, cystic lesions accounted for 44% of lesions, solid 20%, solid & cystic 28% and no definitive mass was identified in 8% of cases. The outcome of the 50 atypical cases was as follows: 5 went to CNB (10%), 8 underwent resection (16%), 26 had clinical diagnoses (54%) and 11 had no follow-up (22%). The diagnoses are provided in Table1. A definitive diagnosis was obtained in all five of five (100%) CNB obtained. The FNA under-diagnosis in all of the 8 resected cases was attributable to low cellularity. Of the 39 atypical cases with a diagnosis rendered, 11 cases (28%) were malignant, the remaining 28 (72%) were benign cysts or non-neoplastic processes.

Table 1: Characterization of 39 cases with follow up

	ACA	IPMN	NE	SCA	LEC	PsC	Inflm	LY	IT
CNB	3			1			1		
Resection	3	3	1		1				
FNA ancillary studies								1	
Clinical impression		9		1		3	7		
Biopsy of other sites	4								1

Key: ACA-adenocarcinoma, NE-neuroendocrine tumor, SCA-serous cystadenoma, LEC-lymphoepithelial cyst, PsC-pseudocyst, Inflm-inflammatory, LY-lymphoma, IT-inflammatory pseudotumor, IPMN- intraductal papillary mucinous neoplasm

Conclusions: In EUS-FNA of the pancreas, the atypical category is more frequently applied to cystic lesions than to solid pancreatic masses. Low cellularity is the most common reason for applying this category, which may be related to operator-dependent sampling issues or the anatomic nature of the lesion. Concurrent CNB is an effective diagnostic adjunct to FNA when possible. Clinicopathologic correlation is essential for optimal management.

402 Pathologic Description of Pap Smears & Biopsies in Gastric Type/Minimal Deviation Endocervical Adenocarcinomas

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Background: Gastric type (GT) mucinous endocervical adenocarcinoma (AD) is an aggressive tumor not associated with human papillomavirus (HPV) infection. It represents a spectrum of mucinous AD of the uterine cervix, including minimal deviation adenocarcinoma (MDA), and characteristically shows cells with voluminous, clear to amphophilic cytoplasm and distinct cell borders. They can be difficult to diagnose accurately on pre-resection biopsy and/or cytology due to their tendency to be well-differentiated histologically, which can lead to delayed treatment. Thus, our goal was to examine cytology (CY), biopsy (BX) and curettage (CU) specimens in cases of GT/MDA and describe the cytomorphologic features.

Design: The CY, BX and CU specimens from patients with resected GT/MDA were reviewed from two institutions. All available slides were reviewed and a detailed morphological analysis was performed.

Results: Ten cases were identified (9 GT, 1 MDA), all patients with CY (9 cervical ThinPrep® Papanicolaou stains, 1 pelvic fluid cytospin Pap stain), 2 with concurrent BX, and one with concurrent CU; with the following diagnoses: AD (6 cervical Paps, 2 BX), suspicious for AD (2 cervical Paps, 1 pelvic fluid, 1 CU), atypical glands of undetermined significance (AGUS) (1 cervical Pap). Cases called AD or suspicious on CY (all GT) displayed anisonucleosis, prominent nucleoli, fine chromatin, dense nuclear membranes and abundant foamy cytoplasm. Cells were columnar or round and in cohesive, crowded 3-D clusters and/or disorganized sheets with occasional naked nuclei. Three of 8 cases had a tumor diathesis. The case of AGUS (MDA) was similar but had scant tumor cellularity and a clean background. The 2 cervical BXs showed infiltrative glands, some with cytoplasmic mucin. Signet ring cells were apparent in one case. Nuclei ranged from small and hyperchromatic to large with prominent nucleoli. One case showed abundant dense eosinophilic cytoplasmic globules. Mitoses and apoptoses were not apparent. The atypical CU specimen (MDA) showed strips of mucinous cells with small bland nuclei with abundant apical mucin, as well as markedly enlarged nuclei with prominent nucleoli and irregular nuclear contours.

Conclusions: The features of GT/MDA on CY often show clusters of cells with prominent nucleoli and abundant foamy cytoplasm. BX and CU can be slightly more difficult, especially in MDA, but careful evaluation of key cytomorphologic features including the cytoplasmic quality and presence of prominent nucleoli can aid in making a timely and accurate diagnosis.

403 A Survey of Practice Patterns of Participants in the College of American Pathologists Interlaboratory Comparison Program in Nongynecologic Cytology (NGC)

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Background: Nongynecological cytology(NGC) practices are expected to expand relative to gynecologic cytology.

Design: The Supplemental Questionnaire: Current Nongynecologic(Non- FNA) Practices in Cytopathology Laboratories(NGSQC) mailed to 2059 laboratories in 2010.

Results: 51% of laboratories responded(1048/2059) where NGC samples were reviewed in non-profit non-training settings by pathologists without specialty training. Cytotechnologists reviewed NGC in 67.4%(675/1002) of laboratories. Annual mean and median volumes of NGC were 1,927 and 858. Laboratories use more than one method to process NGC; cell-blocks are most frequent(930/1029; 90.4%) and are created with centrifugation to pellet (56%;538/961). Direct smears are second most frequent. Discrete staining is preferred to batch staining.

Specimens Preparatory Methods

Specimen	N	% of Laboratories	Methods (%)				
			Smear	Cytocentrifugation	SurePath	ThinPrep	Cell Block
Anorectal	505	48.8	65.0	29.3	14.7	57.8	39.4
Biliary	779	75.3	75	32.5	6.7	49.0	47.8
Body Fluid	992	95.8	50.7	56.5	7.5	51.8	88.8
Bronchial	965	93.2	71.9	45.9	7.7	52.0	69.3
Bronchoalveolar lavage	900	87.0	47.7	49.2	6.9	52.0	67.4
Cerebrospinal	945	91.3	21.1	69.1	4.0	35.3	16.1
Esophageal	835	80.7	71.7	30.5	5.4	49.5	45.0
Gastric	653	63.1	67.7	34.0	6.0	52.1	49.6
Nipple	900	87.0	92.8	17.6	4.1	26.9	16.0
Sputum	963	93.0	60.3	24.5	5.1	46.3	48.2
Urine	983	95.0	18.5	45.1	7.0	53.2	23.1

indicates specimen types and methods used in 1035 reporting laboratories. NGC is used for molecular studies in 34.9%(350/972) of laboratories; flow cytometric immunophenotyping is performed by 55.9%(554/991) and immunocytochemistry(ICC) by 92%(911/991) of responding laboratories. Specimen completion (TAT) is two days or less. Cyto-histological correlation is done by most laboratories concurrently and retrospectively.

Conclusions: The results of this 2010 survey can serve as a basis for future efforts in quality assurance and process improvement in NGC.

404 Cervical Cytology of Stratified Mucin-Producing Intraepithelial Lesions

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Background: Stratified mucin-producing intraepithelial lesion (SMILE) is a rare subtype of adenocarcinoma in situ (AIS) of the cervix. SMILE histologically resembles to a squamous intraepithelial lesion (SIL) but is characterized by stratified columnar neoplastic cells with diffuse intracellular mucin production (Figure 1). Neither the possibility of detection nor the cytologic features of SMILE have been previously reported.

Design: The files in two institutions were searched for any SMILE cases that had corresponding Pap tests within 12 months prior to histologic diagnosis. Pap test slides were reviewed and correlated to the histologic findings and clinical data.

Results: 12 cases of biopsy proven SMILE, occurring in women aged 22-41 (mean 30 years) with a total of 18 Pap tests were identified. Of the 18 Pap tests, reported diagnoses included 4 negative for intraepithelial lesion or malignancy (NILM), 5 atypical squamous cells of unknown significance (ASC-US) or atypical squamous cells, cannot exclude high grade SIL (ASC-H), 2 atypical glandular cells of unknown significance (AGUS), 2 LSIL, 1 HSIL, and 4 HSIL plus AGUS or AIS. In 2 of the AIS cases, SMILE was suggested. Upon retrospective review, atypical glandular cells, misinterpreted as reactive endocervical cells initially, were identified in all 4 NILM and 1 ASC-US (Figure 2). The 11 slides containing AGUS/AIS cells showed dark, crowded groups of cells with enlarged, round to irregular nuclei, course chromatin, inconspicuous nucleoli and moderate amounts of mucinous cytoplasm. Following the diagnosis of SMILE all patients underwent conization. In one case, invasive adenocarcinoma was also present on LEEP excision and hysterectomy was performed. In 7 of 12 cases, HSIL and/or AIS were also present.

Conclusions: SMILE is a diagnosis of relatively young women and it often coexists with significant lesions, such as HSIL, AIS and even invasive carcinoma. Although SMILE could be detected by Pap tests, it was only recognized as AGUS/AIS in 6 (33.3%), and correctly suggested in 2 (11%) cases. A substantial portion of cases were unrecognized. Awareness of this variant of AIS and its unusual cytomorphology on Pap test should be underscored.

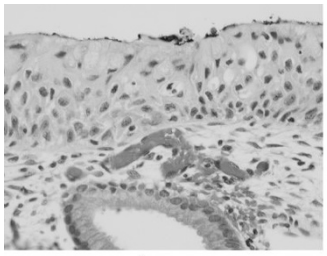

Figure 1

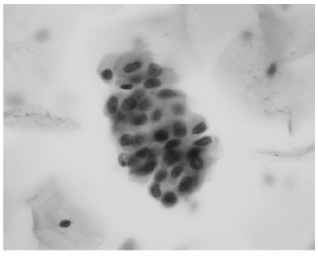

Figure 2

405 The Value of Levels and p16 Immunostains in the Follow-Up of HPV Positive-Biopsy Negative Cervical Lesions

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Background: Current recommendations for ASC-HPV + findings with a (-) biopsy are to repeat cytology at 6 and 12 mos or repeat HPV testing at 12 mos. There are no current recommendations for the immediate assessment of ASC-HPV + and biopsy (-) specimens. We believe sampling and interpretation errors accounts for a significant number of these discrepancies and that re-review of the biopsy may reveal dysplasia, obviating the need for subsequent screening follow-up. The objective of this study is to assess if re-review of (-) biopsies and/or levels or p16 would detect undiagnosed lesions.

Design: We reviewed all ASC pap tests from 10/05-07/11 in which HPV+ patients had (-) follow-up biopsies. These biopsies were blindly reviewed by two pathologists and classified as positive, atypical and negative. Three deeper levels were performed on all (-) and atypical cases and were classified as positive, atypical or negative. p16 immunostains were ordered on atypical and equivocal cases and interpreted according to the CAP/ASCCP Lower Anogenital Squamous guidelines. The follow-up biopsies were handled in a similar manner. The number of cases detected by re-review, levels and/or p16 were calculated in comparison to follow-up biopsies as well as percentage of cases missed due to sampling and interpretation error.

Results: During the study period 2400 of 4199 ASC pap tests were (+). 853 had available biopsies; of these 240 biopsies were (-). On subsequent follow-up, 65 (27%) had a second biopsy and are the focus of this study. Upon review, 10 cases were classified as (+) (8 CIN_1, 2 CIN_2+). 55 cases were (-) and on deeper levels, 6 were (+) (4 CIN_1, 2 CIN_2+) and 2 were atypical. p16 immunostains were done in 15 cases. Of these, 5 were (+) and 10 were (-) or showed non-specific staining. In 2 cases p16 changed the diagnosis and in 3 cases it clarified the level of dysplasia. With reclassification after review, levels and p16, 17 cases were (+) (11 CIN_1, 6 CIN_2+) and 48 (-). Of the 48 (-) cases, squamous epithelium/transitional zone was not present in 41. Including cases that required levels for a CIN_1+ diagnosis, the total number missed due to sampling was 46 of 65 (71%). On a similar review for the follow-up biopsies, 43 were reclassified as (+) and 22 as (-). Of these 43, 17 (39%), including 6/22 (27%) CIN_2+, would have been detected by re-review, levels and/or p16.

Conclusions: Sampling and interpretation errors account for a significant percentage of ASC-HPV +, biopsy negative cases. We recommend re-review, deeper levels and/or p16 to identify the source of this discrepancy.

406 Discordant Pap Tests (HPV Positive Cytology Negative Cotests): A Follow-Up Study of 334 Patients

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Background: As per current ASCCP (American Society for Colposcopy and Cervical Pathology) guidelines, women with HPV positive and cytology negative cotests should either undergo repeat cotesting after 12 months or reflex genotype-specific testing for HPV 16 or HPV 16/18. Immediate colposcopy is not recommended in this cohort of patients, unless they test positive for HPV 16 or HPV 16/18. These guidelines have been revisited recently in 2012. In this study, we examine how far these guidelines have got integrated into our practice and whether the current management strategy appears to be justified.

Design: A retrospective database search was conducted at our institution for the year 2010 to identify women ≥ 30 years of age with Pap tests with negative cytology and positive HPV cotesting results. Clinicopathologic data was accrued for patients tested from January 2010 to June 2010 including subsequent histologic and cytologic follow-up.

Results: A total of 22160 Pap tests (Thin Prep) were performed in the year 2010 in women ≥ 30 years of age. Of these, 20320 women had negative cytology - 20260 of which were cotested with HPV (Hybrid Capture 2). A negative cytology result with a positive HPV cotest was obtained in 794 patients (3.6% of all patients with Pap tests in this age group). Data analysis was performed for patients tested from January 2010 to June 2010. Of these, patients with a documented history of ≥ ASCUS cytology in the previous 2 years were excluded. 334 patients fit the study criteria – average age 44.6 years (range 30-80 years), 231 (69.2%) had cytologic and/or histologic follow-up (follow-up interval of 0.5 to 29 months). 11 patients underwent immediate colposcopy (within 2 months). HPV genotype-specific testing was not performed in any of the patients. CIN2 or above was identified in 8 (3.5%) patients with follow-up - two CIN3 cases and six CIN2 cases, after an interval of 1 to 24 months (average interval 17.9 months) from the index Pap test. None of the patients were diagnosed with invasive carcinoma on follow-up.

Conclusions: Our study reveals that though a substantial number of women ≥ 30 years of age have HPV positive cytology negative cotests, their short term risk of developing CIN3 or above is very low. Henceforth, instead of immediate colposcopy, follow-up with repeat co-testing after 12 months is reasonable in this group of patients.

407 Diagnostic Utility of MIB-1 and p53 in Separating Metastatic Carcinoma of Gynecologic Origin from Benign Epithelial Lesions in Pelvic Washing Cytology Specimens

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Background: Benign reactive conditions such as endosalpingiosis in female peritoneal washings can oftentimes be a diagnostic challenge. Misdiagnosing benign conditions as carcinoma, especially in patients with known ovarian or endometrial carcinoma, can have disastrous implications. Several studies have proposed an immunohistochemical (IHC) profile on these atypical cells but they only demonstrate whether the cells are of epithelial or mesothelial origin. The question still remains whether the cells are reactive or malignant. In this study, we evaluated the utility of p53 and MIB-1 to distinguish reactive endosalpingiosis from metastatic carcinoma under the hypothesis that the reactive condition should have very low proliferative index and lack expression of p53, while metastatic carcinoma might be immunohistochemically positive for p53.

Design: Thirty-seven peritoneal cytology cases including 22 cases of endosalpingiosis and 15 cases of metastatic carcinomas of gynecologic origin were retrieved from our database. Surgical resections of 5 benign fallopian tubes served as negative control. Immunoreactivity for p53 was scored in a four-tiered manner with positive results defined as scores of 1 (<25% cells staining with minimal intensity), 2 (moderately intense staining in 25-50% of cells) and 3 (strong staining in >50% of cells). MIB-1 proliferation index was calculated by counting the number of positive cells in 5 separate high power fields.

Results:

MIB-1 and p53 Reactivity in endosalpingiosis and metastatic carcinoma

	MIB-1	p53
Endosalpingiosis	2%	0% (0/22)
Metastatic carcinoma of gynecologic origin	17%	60% (9/15)
Benign fallopian tube	0%	0% (0/5)

None of our endosalpingiosis cases stained for p53, while 60% of metastatic carcinomas showed diffuse and strong positivity for p53 ($P < 0.001$, chi^2 test). The average MIB-1 count for endosalpingiosis and metastatic carcinoma were 2% and 17%, respectively.

Conclusions: Our results indicated that benign epithelial lesions in peritoneal fluid cytology show low proliferative index and lack p53 expression, while metastatic carcinomas show much higher proliferative index and strong p53 expression. We suggest a panel of MIB-1 and p53 can be very useful in distinguishing benign reactive endosalpingiosis from metastatic carcinoma in pelvic washings, especially in patients with a prior history of uterine or ovarian carcinoma.

408 TelePAPology vs. Liquid-Based Cervical Cytology: A Comparative Study Evaluating p16 Immunohistochemistry and HPV Testing by Hybrid Capture-2 and In Situ Hybridization

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Background: The etiologic role of HPV in cervical carcinogenesis is well established. p16, an excellent surrogate marker of HPV E7 functional inactivation of retinoblastoma gene protein, is a cyclin-dependent kinase inhibitor and a marker of cell cycle dysregulation. The use of p16 immunohistochemistry (IHC) has been advocated as an adjunct method in evaluating HPV presence in cervical lesions. Cellblock (CB) preparations from residual liquid based (LB) pap samples have been shown to be of diagnostic value. We have demonstrated the feasibility of not only utilizing imaging technology to overcome current limitations by digitizing cytologic specimens from CB preparations (Telepap method), but also to the value of CB preparation as a source of material for HPV in situ testing. In this study we evaluated HPV in situ hybridization (ISH) on CB preparations and compare the results with p16.

Design: 311 H&E stained CB slides prepared from CBs (Hologic, Marlborough, MA) from residual LP samples were analyzed. These included ASCUS (141), LGSIL (117), HGSIL (24), AGUS (15), normal (6), ASC-H (5), adenocarcinoma (2), squamous cell carcinoma (1) cases. Telepap slides were obtained using the Aperio imaging system (Vista, CA). HC-2 testing (QIAGEN, Inc, Valencia, CA) and HPV ISH testing (*i*VIEW Blue Detection Kit) (Ventana Medical Systems, Tucson, AZ) and p16 IHC were performed on all CB samples. Test performance characteristics of LP and Telepap samples were compared for diagnostic accuracy and HPV and p16 assay performances.

Results: Telepap virtual slides contained optimal amount of material from all cases. Compared to LP diagnoses, fewer ASCUS and LGSIL cases were diagnosed by Telepap method (124 vs. 141 for ASCUS and 94 vs. 117 for LGSIL cases) and more normal cases were diagnosed (41 vs. 6). The total percentage of HPV+/total HPV tests performed on LP and Telepap specimens were 64% and 66%, respectively. Percent HPV+/total HPV tested ASCUS and LGSIL LP cases were 56 and 96%, respectively and in the Telepap cases in was 51 and 84%, respectively. Percent p16+/total p16 tests performed was 35% in all Telepap samples. The percent +p16 cases in each category was 30% ASCUS, 33% LGSIL and 72% of HGSIL.

Conclusions: The concept TelePAPology is suitable for routine cytology, ISH and IHC testing for HPV and other prognostic markers. HPV ISH and p16 IHC testing is feasible, cost effective and practical. Combination of the two tests would ultimately improve diagnostic accuracy leading to better therapeutic decisions.

409 Thymic Neoplasms: A Study of 59 Cases Diagnosed by Fine Needle Aspiration of the Anterior Mediastinum

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Background: Image-guided fine needle aspiration (FNA) has been used routinely for diagnosing anterior mediastinal lesions. In this study, we retrospectively reviewed 59 cases of thymic neoplasms diagnosed by FNA over a 20-year period and determined the accuracy and clinical utility of this diagnostic technique.

Design: A computerized search of our cytology database was performed for the 20-year period from 1990 through 2010 and all FNAs of the anterior mediastinum diagnosed as thymic neoplasms were identified. All cytology and correlating surgical reports as well as the clinical histories were reviewed and slides from selected cases were re-examined. Nondiagnostic FNA cases without correlating histology were not included in this study.

Results: A total of 59 cases of thymic neoplasm diagnosed by FNA were retrieved and histologic correlation was available for 43 (73%) cases. The FNA diagnoses were classified as: positive in 51 (87%), atypical/hypocellular in 6 (10%) and nondiagnostic in 2 (3%). Fifty-one positive cases included 27 thymomas (53%) and 24 thymic carcinomas (47%). The FNA diagnoses for the 35 of 51 cases (69%) that had histologic confirmation included 18 thymomas (51%) and 14 thymic carcinomas (40%). One case of large cell lymphoma (3%) was misdiagnosed as a malignant thymoma by FNA. For 2 poorly differentiated carcinoma cases (6%), histopathologic confirmation was not obtained due to surgical pathology sampling error. The histologic diagnosis for 6 FNA cases diagnosed as atypical/hypocellular included 1 thymoma, 1 thymic carcinoma, 1 undifferentiated malignant neoplasm, and 1 lymphoma. Two cases with follow-up surgical core biopsies remained inconclusive due to inadequate sampling. In addition, 1 thymoma and 1 thymolipoma were identified on the histologic follow-up of 2 nondiagnostic FNA cases.

Conclusions: FNA is a safe and valuable method for diagnosing thymic neoplasms. In this study, the overall diagnostic accuracy for the FNA diagnosis of thymic neoplasm was high and the misclassification rate was low (3%). When coupled with clinical and imaging findings, FNA should be considered as the biopsy method of first choice for the diagnosis of thymic neoplasms.

410 Metastatic Tumors to the Pancreas Diagnosed by Fine Needle Aspiration: A Multi-Institutional Analysis of 38 Cases

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Background: Most primary malignant pancreatic neoplasms are of ductal origin however non-pancreatic tumors rarely metastasize to the pancreas. Because of their radiologic appearance and sometimes misleading cytology these cancers may simulate primary pancreatic tumors leading to erroneous diagnoses and delayed/inappropriate management. We present our multi-institutional experience with secondary pancreatic tumors diagnosed exclusively by fine needle aspiration (FNA) with/without the use of immunocytochemistry (ICC).

Design: A retrospective review of the pathology departments' archives from December 1988-April 2005 (Emory) and October 2008-June 2012 (Moffitt) yielded 38 cases fitting the criteria for secondary non-hematopoietic pancreatic malignancies. Demographic, clinical, cytologic and diagnostic data were collected.

Results: Patient demographics are summarized in Table 1.

Table 1. Patient Demographics

Males	Females	Age Range	Mean	HOP, Uncinate Process	BOP	TOP	Pancreas, NOS
26	12	23-82	71	18	9	4	7
68%	32%			47%	24%	11%	18%

HOP, head of pancreas; BOP, body of pancreas; TOP, tail of pancreas

There was 1 small cell carcinoma (SCC) of gallbladder and 1 metastatic testicular embryonal carcinoma in a 23 year-old. There were 9 lung cancers (2 squamous, 5 adenocarcinomas, 1 giant cell and 1 SCC); 2/38 (5%) patients had no history of malignancy prior to FNA (1 testicular and 1 lung cancer). ICC supported the diagnosis in 21/38 (55%) cases while morphologic comparison to the prior resection/biopsy supported the diagnosis in 9/38 (24%). Tumor types are summarized in Table 2.

Table 2. Type and Origin of Metastatic Tumors

Tumor Type	Number = n	%
Lung	9	24%
Melanoma	3	8%
RCC	17	45%
Gallbladder	1	2.6%
Intrahepatic duct	1	2.6%
Colon	2	5%
HCC	2	5%
Gastric	1	2.6%
Testis	1	2.6%
Ileal conduit	1	2.6%

RCC, renal carcinoma; HCC, hepatocellular carcinoma.

Conclusions: RCC was the most common metastatic tumor identified on pancreatic FNA, followed by lung carcinoma. The pancreatic head/uncinate process was the most common site of involvement followed by the body. Masses in this location can lead to erroneous radiologic interpretation as pancreatic primaries. However a history of carcinoma (which was given in the majority of our cases) should facilitate accurate diagnosis. In tumors with unusual presentation and obscure cytomorphology a higher index of suspicion is needed to ensure accurate diagnosis and staging. Secondary tumors should always be considered in the setting of pancreatic neoplasms, even in the very young.

411 The Pittsburgh Cervical Cancer Screening Model Indicates That Patient History Significantly Impacts Future Cervical Cancer Risk in Patients with Current Negative HPV Results

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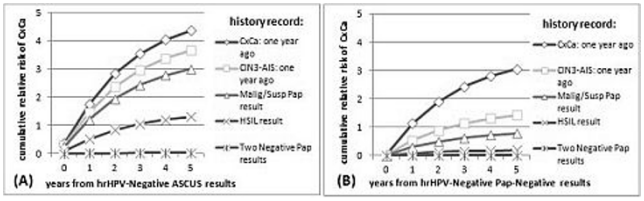
Background: Recent cervical screening guidelines emphasize current high risk (hr) HPV and/or cytology cervical screening as the basis for risk stratification and follow-up. We utilized, the Pittsburgh Cervical Cancer Screening Model (PCCSM) to assess the impact of patient history on subsequent risk for invasive cervical cancer (CxCa) in patients with current negative (neg) hrHPV results.

Design: Utilizing the dynamic Bayesian PCCSM, we estimated 5-year risk projections for CxCa in patients with current cervical screening test (CST) results of either hrHPV-Neg ASCUS or hrHPV-Neg Pap-Neg ("double-negative") results (DNR). Risk assessment calculations took into account both current (CST) as well as previous patient's results. Our database included 696,172 liquid based cytologies, 155,246 adjunctive hrHPV tests, and follow-up surgicals from approximately 10% of 271,029 patients. Vaginal cytologies and patients with only one cervical cytology and no follow-up were excluded.

Results: Cervical cytologies (525,689) from 156,534 patients (ave age μ=41.1) were included. Patients with current hrHPV-Neg ASCUS and history of recent histopathologic CxCa were associated with the highest subsequent risk for development of either CxCa, followed by recent prior histopathologic CIN3/AIS. Prior history of either malignant/suspicious cytology or HSIL were associated with the next highest estimated risk for subsequent CxCa among patients with current hrHPV-Neg ASCUS. Similar trends were noted for patients with current DNR, but at lower risk levels. Figure 1 shows cumulative relative risk of CxCa in patients with (A) current hrHPV-Neg ASCUS (B) current DNR.

Conclusions: PCCSM shows that higher future risk estimates were associated with current hrHPV-Neg ASCUS than with current DNR. This may be due to risks associated with undetected latent persistent hrHPV infections. The PCCSM indicates that patient history should be taken considered when developing individualized CST recommendations.



412 Evaluation of Myeloid Leukemic Effusions in Cytopathology

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Background: Current literature distinguishing primary leukemic effusions (PLE) from peripheral blood (PB) contamination in acute myeloid leukemia (AML) is limited. We attempt to evaluate a series of myeloid effusions, and to determine if true PLE could be distinguished from PB contamination.

Design: In a 10 year archival search, we identified 14 effusions (body fluids excluding CSF) from 9 patients with final diagnosis of atypical, suspicious or positive for leukemic blasts. Effusion preparations were reviewed and correlated with clinicopathologic variables and diagnostic material for primary myeloid neoplasm (2008 WHO Criteria).

Results: Primary myeloid neoplasms included 4 AML with myelodysplasia related changes, 3 therapy related myeloid neoplasms, 1 AML not otherwise specified and 1 AML unclassifiable by available material. Monocytic differentiation was present in 5 cases. At presentation with effusion, median patient age was 60 years with a 1.25:1 male:female ratio. Table 1 includes selected case characteristics and classification of these effusion. 2 cases had follow up tissue biopsy: case 5 showed pleural myeloid sarcoma and case 2 reactive pericarditis.

Table 1. Effusion characteristics

Case	Effusion site	% peripheral blood blasts at time of effusion	Days from diagnostic specimen to effusion	Effusion blast # (1-3)*	Effusion maturing granulo-cytes	Effusion red blood cells	Apparent Classification of Effusion
1a	Peritoneal	0 (9% monocytes)	449	1, but 2-3 immature monocytes	Yes	Moderate	Contamination
1b	Peritoneal	0 (2% monocytes)	440	1, but 2-3 immature monocytes	Rare	Scant	Indeterminate
2a	Peritoneal	11	-1	2	Yes	Scant	Leukemic
2b	Pericardial	9	0	1	Many	Abundant	Contamination
2c	Pleural , right	5	1	1	Yes	Moderate	Contamination
2d	Pleural, left	5	1	1	Yes	Moderate	Contamination
3	Pleural, right	3	783	1	Yes	Moderate	Contamination
4	Pleural, left	45	672	3	Rare	Abundant	Contamination
5	Pleural, left	0	-1	3	No	Abundant	Leukemic
6a	Pleural, right	2	33	3	Rare	Abundant	Leukemic
6b	Pleural, left	2	33	3	Yes	Abundant	Leukemic
7	Pleural, NOS	0	6	1	Yes	Abundant	Indeterminate
8	Pleural, NOS	18	166	3	Yes	Abundant	Contamination
9	Pleural, NOS	0 (71% monocytes)	20	2	Yes	Abundant	Contamination

*1=few, 2=moderate number, 3=many

Conclusions: Myeloid neoplasms, particularly with monocytic differentiation, may be detected in PLE or following contamination with blasts in PB. PLE tend to have increased blasts in fluid, but low PB blast counts, and can usually be separated from PB contamination by correlation with PB findings.

413 DNA Qualitative and Quantitative Comparison of Alcohol Fixed Cytology Versus Formalin Fixed Tissue for PNA Clamp Real-Time PCR Detection of KRAS, EGFR and BRAF

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Background: Formalin-fixed paraffin embedded tissue (FFPE) is the FDA-approved substrate for polymerase chain reaction (PCR) detection of mutations in BRAF and KRAS, and is commonly used for EGFR testing. We evaluate the quality of the desired DNA required for adequate molecular analysis in alcohol fixed cytology specimens, compared to FFPE.

Design: At UMASS, 20 surgical FFPE (8/2009-7/2012) and 20 alcohol fixed cytology cell blocks (1/2011–6/2012) from multiple organ sites were analyzed. Tumor was macrodissected from slides. DNA was extracted (~[/underline]10ng) and tested by spectrophotometry, multiplex endpoint QC-DNA assay and PNA clamp real-time PCR for BRAF, KRAS and EGFR (see Table 1). Peripheral blood DNA was used as the quality gold standard. The delta CT was calculated by subtracting the patient DNA CT value from the peripheral blood DNA.

Results: We evaluated the following parameters: DNA yield, OD 260/280 ratio and maximum amplicon size. The range and average values are shown:

Table 1. DNA results.

Parameter	Cytology	Surgical
EGFR	9	6
EGFR+KRAS	1	1
EGFR+KRAS+BRAF	0	1
KRAS	0	3
KRAS+BRAF	10	9
Average DNA yield (range)	1.48 (0.37-4.35)	1.48 (0.10-4.275)
Average OD260/280 (range)	1.92 (0.94-2.37)	1.98 (1.66-2.46)
Average amplicon size (range)	448 (QNS/100-500)	444 (QNS/300-500)
Average Delta CT	-1.5238	-2.82812

A delta CT equal to (or greater than) zero is equivalent to (or better than) the optimal DNA quality of the control peripheral blood. FFPE samples showed a slightly higher average delta CT by 1.3 PCR cycles compared to cytology cell blocks suggesting higher DNA quality for cytology samples. The OD260/280 is a measure of DNA purity with an optimal range of 1.8-2.2. No obvious differences between fixation methods were observed for quantity, average amplicon length, or OD 260/280.

Conclusions: The DNA purity and quantity of surgical and cytology specimens appear to be equivalent. DNA fragmentation based on PCR amplicon size shows the two fixation methods are virtually the same. The average deltaCT was larger for surgical specimens. The latter may be the result of a small cohort or, if considered real, may be attributable to formalin induced cross-linking with resultant retardation of PCR amplification. These findings validate the use of alcohol fixed cytology as a suitable and comparable specimen type for molecular testing.

414 Alcohol and Formalin-Fixed Cytology Specimens Offer an Effective Alternative to Formalin-Fixed Tissue for ALK Break-Apart FISH Testing in Lung Adenocarcinoma

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Background: For most patients with lung nodules, multiple molecular studies are often requested to determine targeted therapeutic options. In the case of crizotinib therapy, the predicted response is dependent on detection of ALK rearrangement using the gold standard fluorescence in-situ hybridization (FISH). While the current FDA guidelines require formalin fixed paraffin embedded (FFPE) tissue sections, our study validates the utility of alcohol and formalin-fixed cytology specimens.

Design: As part of our institution's (UMMHS) routine clinical practice, between November 2010 and October 2012, ALK FISH was performed on 116 lung adenocarcinoma cytology or surgical specimens. All specimens used papanicolaou stain, with integrated cytomorphology and FISH analysis, through the BioView automated Imaging system. FISH slides were scored according to the package insert. As part of the validation, 26 specimens were run in duplicate against the standard methodology carried out at reference laboratories. All slides on file (40 cytology and 38 surgical cases), were reviewed by two pathologists to assess the predominant morphologic patterns.

Results: Our cohort included 116 tumors of which 53% (62) were cytology and 47% (54) were surgical specimens. The mean age was 66 years. The 26 specimens ran in duplicate were concordant with the referral lab result. Eighteen failures were due to: (1) insufficient cells, (2) no hybridization signals, and/or (3) cancellation of the test or positivity of EGFR or KRAS. The cytology specimens included 44% (27/62) FNA's and 56% (35/62) fluids, washes and brushes. Of the surgical specimens, 57% (31/54) were biopsies. All three ALK positive cytology cases (5%, 3/62) were pleural fluids, submitted as formalin-fixed conventional cell blocks, and 2 displayed a micropapillary pattern. Of the ALK positive surgical cases (6%, 3/54), 2 showed a predominantly mucinous (67%) pattern. Failed testing in the conventional cell blocks was due mainly to lack of hybridization signals (4/19), and due to insufficient cellularity in the alcohol fixed rapid cell blocks (3/28).

Conclusions: Our study validates the utility of alcohol or formalin fixed cytology specimens for ALK FISH testing. In the new climate of cost efficient healthcare with an emphasis on less invasive procedures, ALK FISH testing on cytologic specimens may limit the need for tissue biopsy and offers invaluable therapeutic options for patients in which tissue can not be obtained.

415 Common and Rare EGFR Mutations. The Challenge of Their Cytological Detection

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Background: Lung cancer represents the third most common cancer and the first cause of cancer mortality.EGFR mutation is referred in about 30-40% of patients with lung adenocarcinoma or mixed squamous-adenocarcinoma type and is essential for therapeutic decision.EGFR mutations are located within exons18 to 21 of the kinase domain, mainly in-frame deletions in exon19 and/or point mutations in exon 21.The detection of rarer mutations in exons18 and 20 can be performed by direct sequencing even if the quantity of neoplastic cells,obtained for cytology,is rather limited. The objective of this study was to compare the rate of detection of EGFR mutations in cytological samples to that observed in histological samples.

Design: A total of 893 lung cancer patients(491 female and 402 male),with a median age of 52 y-old were studied for EGFR mutations using Sanger-sequencing.The cohort branched into 790 histological samples(small biopsies) and 103 cytological cases.The histological diagnoses were:457 adenocarcinomas, 407 non-small cell carcinoma and 29 squamous cell carcinomas.

Results: A total of 164(18.3%)mutations were found,including common mutations in exons 19 and 21 and rare mutations in exons 18 and 20.The common mutations were in-frame deletions of exon 19(88/164;53.6%)and substitutions in exon 21(56/164;34.1%). The rare mutations corresponded to substitutions in exon 18(7/164;4.2%) and other different mutations (including insertions, deletions and substitutions)in exon 20(13/164;7.9%).Table 1 summarizes the findings of EGFR mutations in the two types of samples and there is no statistically significant difference between the rates of detection of mutations in exon 19 and 21,and exon 18 and 20 in cytological and histological material(chi-square value:4.45;p-value:0.1079).

Frequency of EGFR mutations in cytological and histological samples of lung cancer patients

	Cytological samples	
EGFR-wild type	85 (82.5%)	644 (81.5%)
EGFR common mutations	13 (12.6%)	131 (16.5%)
EGFR rare mutations	5 (4.8%)	15 (1.8%)

Conclusions: These results show that common and rare EGFR mutations can be feasibly detected on routine cytological specimens,with detection rates comparable to those obtained in histological material.In this perspective, the use of cytology for molecular analysis may help in selecting a "personalized" therapy and in providing guidance for switching treatment especially in those patients with metastatic and inoperable diseases.

416 The Sequential Application of Immunocytochemistry, BRAF-1 and N-RAS Mutation Analysis Identifies Malignant Follicular Thyroid Neoplasms on Liquid-Based Cytology

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Background: Fine-needle aspiration cytology (FNAC) is an important tool for evaluating thyroid nodules but up to 20% of cases may result in an indeterminate diagnosis (Follicular Neoplasm – FN and Follicular Lesion of Undetermined Significance – FLUS). Some molecular alterations of specific pathways involved in the oncogenic process, namely *BRAF and RAS* mutations, are referred as markers of malignancy for papillary carcinoma (PTC). *BRAF* mutations have been identified in about 50% of PTC, mostly classic variant, while studies have demonstrated that RAS point mutations may also occur in thyroid carcinomas. In indeterminate and suspicious for carcinoma thyroid lesions (SC) we studied a panel including Immunocytochemistry (ICC) and *BRAF* mutational analysis followed by *N-RAS* in *BRAF* wild-type (wt) cases.

Design: In 2011-2012, 20 cases diagnosed as FN/FLUS (9 Benign and 11 malignant at histology) and 37 SC (7 Benign and 30 malignant lesions) processed with liquid-based cytology (LBC, ThinPrep, Hologic, USA) were diagnosed at the Catholic University of Rome (Italy). All 57 cases underwent HBME-1 and Galectin-3 and mutation analysis of *BRAF*. The 46 cases negative for *BRAF* underwent *N-RAS* codon 61 mutation analysis. ICC as well as DNA extraction were performed on LBC-stored material.

Results: Out of 57, 36 cases (11 FN/FLUS and 25 SC) showed an ICC positivity, 11 of them 25 SC showed a *BRAF* (44%). Out of the 46 *BRAF*-wt cases, 3 had N-*RAS* mutation (6%). In FN/FLUS all ICC positive cases resulted PTC whereas 2 cases showed a *N-RAS* mutation. In the 30 malignant SC, ICC resulted positive in 84.6% with 36% *BRAF* and 1 *RAS* mutations. All 3 *RAS*-mutated cases resulted as Follicular variant of PTC (FVPTC).

Conclusions: The sequential application of ICC and mutational analysis on LBC may enhance the accuracy of FNA and its cost-effectiveness. ICC identifies more malignancies in the FN/FLUS group, especially when supported by *RAS* analysis, whereas cases negative for ICC can benefit of a strict follow-up instead of surgery. In SC, *BRAF* and *RAS* represent strong indicators of malignant outcome and may warrant a more aggressive surgical approach.

417 Cytologic Features of Atypical Cervical Cytology Samples with and without *TERC* Copy Number Changes

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Background: Genomic markers, such as chromosome (chr) aneuploidy and/or amplification of the telomerase RNA gene (*TERC*), are found in the vast majority of high-grade squamous intraepithelial lesions (SIL) but in only a small subset of low-grade SIL lesions. *TERC* gene profiling shows promise in distinguishing genomically complex atypical lesions from those with just atypical cytologic features. Combining *TERC* FISH with cytology, we compared morphologic features in cervical samples that had *TERC* copy number changes with those that lacked them.

Design: *TERC* FISH analysis was performed on 97 liquid-based cervical samples, prepared by the ThinPrep (n = 43) or the SurePath method (n = 54), with diagnosis by the Bethesda system. Percentages of lesional cells were detemined by a cytopathologist as the proportion of atypical cells compared to total epithelial cells in Pap stains. Genomic status of the *TERC* loci was evaluated using a FISH probe at chr band 3q26 (hTERC, Kreatech), and normalized with an alpha satellite probe (3q11, Kreatech). Specificity of probe binding was confirmed by analysis of a subset of cases with a different 3q probe (Vysis). 3q26 and 3q11 signals were quantified using fluorescence microscopy by manual count, and with automated microscopy (Metafer 4, MetaSystems) in a subset.

Results: *TERC* amplification, identified as either chr 3 polysomy or gene-specific amplification, was found in 2/2 of CIS (100%), 25/27 of HSIL (92.6%), 8/26 of ASC-H (30.7%), 2/15 of LSIL (13.3%), and 0/22 negative cases. The two HSILs without *TERC* amplification showed less than 1% and 2-3% lesional cells, with the former case showing normal cytology on follow-up. All *TERC*-amplified HSIL cases had at least 3% cytologically lesional cells and the percentage of lesional cells identified by FISH and cytology review were well-correlated (R= 0.53), with cases with the most lesional cells more frequently showing *TERC* amplification. Among ASC-H lesions, there was a trend towards more cytologically lesional cells in *TERC*-amplified cases versus those with normal FISH (median 4% vs. 1%). The cases with *TERC* amplification were more likely to include lesional cells in small sheets and clusters.

Conclusions: *TERC* copy number changes were found in >90% of HSILs, including all that had at least 3% lesional identified by parallel cytologic counts. Correlates in those ASC-H cases that had *TERC* amplification included a higher percentage of lesional cells, and their presence in small sheets or clusters. These findings show promise in refining the morphologic features of atypical cytology cases that genetically resemble HSIL.

418 Cytologic Diagnosis and Classification of Indolent B-Cell Lymphomas: Results of a 3-Year Institutional Review with Implications for Reporting

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Background: The cytologic diagnosis and classification of indolent B-cell non-Hodgkin lymphomas (B-NHL) is considered a challenging area. A range of reported sensitivities and specificities result in clinical uncertainty regarding treatment decisions based on fine needle biopsy (FNB) alone; a situation increasingly encountered with endoscopic ultrasound-guided sampling. We reviewed the records of our institution with the goal of defining specimen characteristics required to render diagnoses according to the WHO classification.

Design: Laboratory records from 2009-2011 were searched for FNB samples with diagnoses or suspected diagnoses of indolent B-NHL (follicular lymphoma, mantle cell lymphoma, marginal zone lymphoma, small lymphocytic lymphoma, lymphoplasmacytic lymphoma). Diagnoses, ancillary studies, prior lymphoma history and concurrent/subsequent histological specimens were tabulated. For cases without WHO diagnosis any factors limiting assessment were recorded from the pathology report. GraphPad Prism 5.02 was used for statistical analysis.

Results: 287 cases (231 patients) were identified associated with 105 histologic specimens. A diagnosis according to the WHO classification was rendered in 71.1% (204/287) by cytology. Of 83 cases either lacking subclassification (n=54) or diagnosed as suspicious (n=29), 41 (49.4%) were inconclusive by morphology and immunophenotype, 26 (31.3%) were low in cellularity/hemodiluted, 7 (8.4%) showed equivocal light chain restriction, 6 (7.2%) were submitted in alcoholic fixative and 3 (3.6%) were necrotic. Among lymphoma cases inconclusive by FNB and diagnosed by histology (n=24), an increased proportion of marginal zone lymphomas was found (n=7; 29.2%). 6 of 7 cases with equivocal light chain restriction were confirmed as lymphoma. Cytology-histology agreement was 97.3% when discrepancies due to grading of follicular lymphoma were excluded from the analysis. Ancillary testing by immunophenotyping ($p=0.0001$) and fluorescence in-situ hybridization ($p<0.0001$) was significantly associated with subclassified diagnoses.

Conclusions: Cytologic examination of cellular, non-hemodiluted samples in combination with ancillary studies permits a diagnosis according to the WHO classification to be rendered in the majority of cases. Caution is warranted in reporting samples with equivocal features by morphology and ancillary testing. The adequacy of the sample in terms of completeness of required investigations should be stated in the report indicating the certainty of diagnosis.

419 Role of Molecular Analysis on Classification of Pancreatic Cystic Lesions (PCLs) without Definitive Cytological Diagnoses

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Background: The clinical management of patients with PCLs has relied on pathologic diagnoses. Non-mucinous PCLs are usually followed clinically, while some mucinous PCLs require surgical resection. Enodoscopic ultrasound-guided fine needle aspiration and cytological examination are the predominant methods used to evaluate the PCLs. However, a definitive cytological diagnosis(non-mucinous vs. mucinous) can not be rendered in some cases due to the lack of diagnostic cells in the specimen. Recently, molecular analysis of cyst fluid from PCLs has been available (PathFinderTG®, Pittsburgh, PA). The purpose of this study is to determine if molecular analysis would be useful to further classify PCLs which do not have a definitive cytological diagnosis.

Design: Between January 2006 and September 2012, we identified 87 PCLs which met the study criteria. Molecular analyses looking for quality and quantity of DNA, loss of chromosome homogeneity and K-ras mutations,were reviewed and the cases were further classified into non-mucinous, mucinous and malignant. Correlation with the clinical follow-up and surgical pathology of all cases was performed.

Results: Of 87 cases, 82 (94.3%) cases were able to be further classified into non-

mucinous (50, 60.1%), mucinous (30, 36.5%) and malignant (2, 2.4%) by molecular analysis. In 50 non-mucinous cases, 9 cases had surgical resection and 20 cases had clinical follow-up. Of 9 resected cases, 4 were serous cystadenoma (SCA) and 5 were mucinous neoplasm (2 mucinous cystic neoplasm (MCN) and 3 IPMN). In 30 mucinous cases, 8 cases had surgical resection and 15 cases had clinical follow-up. Of 8 resected cases, 7 were mucinous neoplasm (4 MCN and 3 IPMN) and 1 was SCA. Of 2 malignant cases, one was diagnosed as mucinous adenocarcinoma on a repeat FNA and the other one was suspicious for malignancy on follow-up image study. Using the diagnoses of the 17 surgical resection specimens as gold standards, the sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) of molecular analysis were 66.7%, 58.3%, 44.4%, 87.5% for non-mucinous PCLs and 58.3%, 80%, 87.5%, 44.4% for mucinous PCLs, respectively. The overall diagnostic accuracy of molecular testing was 64.7%.

Conclusions: Molecular analysis of PCLs cyst fluid has some diagnostic value for cases without definitive cytological diagnosis. Molecular analysis has better sensitivity and NPV for non-mucinous PCLs and better specificity and PPV for mucinous PCLs. However, a multidisciplinary approach is needed in the management of these patients.

420 Aberrantly Elevated Galectin-3 and Carbohydrate Antigen Tn Expression in Fine Needle Aspiration Specimens of Pancreatic Ductal Adenocarcinoma but Not Neuroendocrine Tumors

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Background: Galectin-3, a beta-galactoside-binding protein, has been implicated in tumorigenesis and tumor progression. However, its impact on pancreatic ductal adenocarcinoma (PDAC) remains controversial, ranging from tumor-suppressive to tumor-promotion properties and negative prognostic effects. Although Galectin-3 was shown to promote metastasis in PDAC cell lines, clinical evidence is still lacking. In addition, its applicability in pancreatic fine needle aspiration specimens has never been tested. Tn, a mucin-like protein glycan, is aberrantly expressed in malignant neoplasms, while minimally expressed in normal tissues. The functional role of Tn and the mechanism by which Tn promotes tumorigenesis remains unclear. We investigated the expression of Galectin-3 and Tn in PDAC, pancreatic neuroendocrine tumors, and non-tumor pancreatic tissues, to evaluate their potential diagnostic values.

Design: Galectin-3 and Tn levels were evaluated in 46 PDAC, 5 neuroendocrine tumors, and 8 non-neoplastic pancreatic fine needle aspiration (FNA) cell blocks (CB) by immunohistochemistry. Membranous and granular cytoplasmic staining of Galectin-3 or Tn (3+ and >10% cells) were considered positive. The data were analyzed by statistical analysis.

Results: Forty-four and 43 of 46 PDAC CB demonstrated tumor-specific Galectin-3 positivity (sensitivity 95.7%; specificity 87.5%; p<0.001) and Tn staining (sensitivity 93.5%; specificity 87.5%; p<0.001), respectively. In contrast, only 12.5% (1/8 and 1/8) non-neoplastic cases showed focal moderate Galectin-3 or Tn staining (2+, p<0.001). Furthermore, no positivity for Galectin-3 or Tn was detected in neuroendocrine tumors.

Pancreatic FNAs (number)	Galectin-3 +++		Tn +++	
PDAC (46)	44 (95.7%)		43 (93.5%)	
Neuroendocrine tumors (5)	0 (0%)		0 (0%)	
Non-tumor (8)	1 (12.5%)		1 (12.5%)	
Total (59)	45	P<0.001	44	P<0.001

Conclusions: Both Galectin-3 and Tn are uniquely and highly expressed in PDAC (p<0.001), but not in neuroendocrine tumors or non-tumor tissues. This suggests that Galectin-3 and Tn could be specific markers representing epithelial neoplasms but not neuroendocrine tumors of pancreas, and further advocate their potential diagnostic and prognostic roles in the cytological work up of pancreatic tumors.

421 Fine Needle Aspiration Is a Valid Tissue Source for EGFR and KRAS Molecular Testing in Lung Cancer: A Comparison Study with Core Needle Biopsy

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Background: There is limited data in the literature comparing fine needle aspiration (FNA) biopsy with core needle biopsy (CNB) for molecular testing. In this study, we compared FNA vs. CNB for mutational analyses of EGFR and KRAS in lung cancer patients.

Design: We retrospectively reviewed the reports generated by clinical requests for EGFR and KRAS mutational analysis in lung cancer cases in the Department of Pathology from 2009 and 2010. Specimens with diagnoses of adenocarcinoma, non-small cell carcinoma-favor adenocarcinoma, or non-small cell carcinoma-NOS were included in this study. There were 210 FNAs and 509 CNBs with molecular testing results. Of these, 172 FNAs and 178 CNBs were performed in-house. FNA and CNB cases were compared with respect to specimen adequacy for testing (i.e. sufficient amount and purity of tumor to submit to the molecular lab), failure of PCR amplification (PCR failure in one or more exons/codons in the absence of a mutation detected in the others), and frequency of detected mutations. Molecular testing was performed in the Molecular Diagnostic Lab using PCR-based sequencing of EGFR exons 18-21 and KRAS codons 12, 13, and 61. Fisher's exact tests were used to assess the association between categorical variables.

Results: Similar fractions of FNAs and CNBs were deemed adequate for testing, but CNBs showed more failures in PCR amplification. Informative results therefore were obtained more often in FNAs, but not significantly (P=0.13) (Table 1). The frequencies of EGFR/KRAS mutations were similar in both groups, among all cases, and among the subset of tumors with known TTF-1 status (Table 2). Among the cases in which the TTF-1 status were known (60.5% of FNAs and 71.7% of CNBs), TTF-1 positive tumors accounted for 80.3% of FNAs and 80.6% of CNBs.

Table 1: Specimen Adequacy and DNA Insufficiency Rates for EGFR/KRAS Molecular Testing, FNA vs. CNB (In-house cases only)

	FNA (%)	CNB (%)	P-value
Adequate for testing	172/197 (87.3)	178/201 (88.6)	0.76
DNA insufficiency	16/172 (9.3)	32/178 (18.0)	0.05
Informative results	156/197 (79.2)	146/201 (72.6)	0.13

Table 2: Frequencies of EGFR/KRAS Mutations, FNA vs. CNB (Combined in-house and outside cases)

	EGFR		KRAS	
	TTF1+	Total	TTF1+	Total
FNA (%)	25/93 (27)	56/238 (24)	22/91 (24)	54/222 (24)
CNB (%)	62/237 (26)	88/412 (21)	53/241 (22)	102/428 (24)
P-value	0.89	0.56	0.66	0.92

Conclusions: FNA is comparable to CNB in providing tissue for EGFR and KRAS mutational analysis by PCR-based sequencing in lung cancer.

422 Epstein-Barr Virus (EBV)-Associated Pleural Effusions: Clinical Features, Cytomorphologic Characteristics, and Flow Cytometric Immunophenotyping

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Background: Pleural effusions (PEs) are frequently encountered specimens in cytopathology. Their etiology varies, and a percentage of PEs remain unexplained despite an intensive diagnostic workup. EBV is ubiquitous in the human population where it establishes lifelong latency after the initial infection. One previous study documented a 40% prevalence of EBV-DNA in unselected PEs. Our aim is to characterize the clinical and cytomorphologic features of EBV-associated PEs (EBV-PEs), which have not been previously described. Flow cytometric immunophenotyping was also performed for lymphocytic PEs.

Design: A database search was performed for PE cases with EBV-DNA identified in the fluid by real-time quantitative PCR in a period from 1/1/2007 to 8/30/2012. The corresponding fluid cytology and fluid chemistry data were reviewed, and the patients' demographic data and clinical features were recorded.

Results: A total of 20 cases (18 patients) of EBV-DNA positive PE were found, and all had corresponding cytology specimens. All PEs were exudates based on chemical analysis. All patients (13 males, 5 females; average age= 64.6 years) had a history of lung transplantation, and most presented clinically with shortness of breath during post transplant follow-up. 4 patients expired within 3 months following the thoracentesis. The median EBV-DNA level was 530 copies/mL (range: <100 – 248,300). Cytologically, lymphocytosis was present in 12 (60%) fluids, characterized by a polymorphous lymphoid population with variable numbers of reactive large lymphocytes. Scattered apoptotic lymphocytes and rare mitotic figures in lymphocytes were identified in 3 cases. Mesothelial cells varied in number and some cases showed reactive atypia. Flow cytometric analysis revealed no monoclonal population or aberrant T cell antigen expression. The lymphocytes were predominantly T cells with the CD4/CD8 ratio varying from 10:1 to 1:2 (reversed ratio).

Conclusions: Although rare, EBV-PEs can be seen in transplant patients. In the absence of an alternative diagnosis to explain the nature of the PEs in patients with a history of lung transplant, EBV-PEs should be included in the differential diagnosis especially when lymphocytosis is present in the fluid.

423 Implantable Port System Cytology for Ovarian Cancer To Evaluate Chemotherapy Response

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Background: Neoadjuvant chemotherapy (NAC) followed by interval debulking surgery (IDS) for advanced ovarian cancer has been shown to be not inferior to primary debulking surgery. However, appropriate number of chemotherapy cycles in NAC are controversial. Clinically, we have evaluated chemotherapy response only using tumor marker for ovarian cancer, CA 125, and radiographic study. In this prospective pilot study, we evaluated morphologic change of cancer cells in implantable port system (IPS)-cytology for chemotherapy response and the relation between IPS-cytology and CA 125.

Design: Patients eligible for this study were those who had unresectable residual disease at the initial surgery. At the initial surgery, the IPS was placed in patient's abdominal wall. To obtain IPS-cytology, 500ml of saline was infused. More than 20ml of saline was collected for cytological evaluation. We performed IPS-cytology every 3-4 weeks during neoadjuvant chemotherapy done. IDS was performed in principle after patients had achieved negative IPS-cytology. We evaluated morphologic change of cancer cells in IPS-cytology during NAC. The morphologic change was compared to CA 125.

Results: Twenty-seven patients were enrolled into this study. All patients had extensive stage T3c disease. Median CA 125 value was 1133 IU/ml in all patients. Five patients had progression disease before IDS was performed. Twenty-two patients received interval debulking surgery after NAC. We observed as following morphologic change of cancer cells in IPS-cytology. At initial surgery, numerous large clusters of cancer cells were observed in IPS-cytology. The clusters were arranged in papillary aggregate. After several chemotherapies were done, papillary clusters had arranged in decreasing papillosity and the number of cancer clusters decrease and the size became smaller. Some cancer clusters had arranged in sheets patterned. Then, cancer clusters were arranged smaller with scant cytoplasm. The cancer cells showed isolated with some degree of degeneration and some nuclei enlarged, subsequently. After isolated cancer cells were observed, we found negative IPS-cytology. Twenty-one of 27 patients had negative IPS cytology. Median period of change to negative IPS-cytology was 19.6 weeks from date of starting chemotherapy, while median period of change to less than 35 IU/ml in CA 125 was 10.1 weeks.

Conclusions: This study revealed patients receiving neoadjuvant chemotherapy achieved negative IPS-cytology exclusively after isolated cancer cells were observed. IPS-cytology are effective to evaluate direct response of NAC for advanced ovarian cancer.

424 Squamous Intraepithelial Lesion of Intermediate Grade: Cytologic-Histologic Correlation and Comparison of Positive Predictive Values

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Background: Within the 2001 Bethesda Reporting System (TBS) for Cervical Cytology, Squamous Intraepithelial Lesion of Intermediate Grade (SIL-NOS) is a diagnostic category that is only briefly defined. Despite this, the recent literature is replete with articles about SIL-NOS, with reported positive predictive values (PPV) for CIN 2+ ranging from 23-65%. We present our data regarding SIL-NOS, and compare its histologic correlation with those of other Pap test diagnoses.

Design: Between 2002-2011, 1,026,470 Pap tests were examined, including 67% SurePath, 32% conventional, and 1% ThinPrep preparations. Subsequent correlating biopsies were categorized as either negative, CIN 1 (low grade dysplasia/condyloma), and CIN 2+ (high grade dysplasia/cancer). As defined in TBS, a diagnosis of SIL-NOS was rendered in the presence of atypical squamous metaplastic cells or keratinized dysplastic cells, that raised the possibility of, but were not diagnostic for HSIL. Other diagnostic criteria included the predominance of LSIL cells with only rare higher grade cells. The Chi-Square test was employed for statistical analysis.

Results: 43,862 cases (4.3%) were flagged as abnormal, including 1.5% ASCUS, 0.1% ASC-H, 1.8% LSIL, and 0.2% HSIL results. SIL NOS was diagnosed in 2,475 (0.2%) cases. 23,243 correlating biopsies were examined (Table 1). The PPV for CIN 2+ for SIL-NOS (42%) was intermediate between that of LSIL (12%) and HSIL (69%), and differed significantly (P<0.01). The patients' ages and duration from Pap smear to biopsy did not vary between the diagnostic categories. No statistically significant differences were noted in SIL-NOS PPVs between conventional and SurePath methods (P=0.11) and amongst pathologists (P=0.97). Furthermore, these PPV values showed no significant variation over the 10 year period (P=0.27).

Conclusions: SIL-NOS is a distinct diagnostic entity from LSIL and HSIL, which is reproducible between pathologists and over time. Further study of SIL-NOS including examination of clinical relevance, and more detailed characterization within TBS, is warranted.

Abnormal Pap Smear Cytologic and Histologic Correlation (2002-2011)

Cytologic Diagnosis	No. Cases	Histologic Diagnosis		
		Negative	CIN 1	CIN 2+
ASC-US	6032	3603 (60%)	1337 (22%)	1092 (18%)
ASC-H	974	425 (44%)	87 (9%)	462 (47%)
LSIL	12353	6817 (55%)	4104 (33%)	1432 (12%)
HSIL	1907	504 (26%)	92 (5%)	1311 (69%)
SIL-NOS	1977	757 (38%)	387 (20%)	833 (42%)
Total	23243	12106 (52%)	6007 (26%)	5130 (22%)

425 The Bethesda System for Reporting Thyroid Cytopathology: A Four-Year Single Academic Institution Experience

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Background: The Bethesda System for Reporting Thyroid Cytopathology (BSRTC) is a standardized reporting system for classifying thyroid fine-needle aspiration results comprising of 6 diagnostic categories with unique risks of malignancy and recommendations for clinical management. Our objective was to report our past 4-year experience with the BSRTC, review its distribution and evaluate its efficiency based on the cytologic-histologic correlation.

Design: A total of 12,930 thyroid nodules undergoing FNA were examined at our institution between January 2008 and December 2011. All FNAs were classified prospectively into unsatisfactory, benign, FLUS/AUS (follicular lesions or atypia of undetermined significance), follicular neoplasm (FN), suspicious for malignancy, or positive for malignancy. The Cyto-histologic correlation was recorded.

Results: Table 1 summarizes the diagnostic frequencies.

4 Years of the BSRTC Diagnostic Frequencies (%)

	2008	2009	2010	2011	Total 2008-11
Non-Diagnostic	11.19	11.04	8.71	7.13	9.44
Negative	73.53	71.28	73.05	76.66	73.75
FLUS/AUS	4.27	4.36	7.10	5.82	5.14
FN	5.30	5.60	4.21	3.11	4.50
Suspicious	1.34	1.37	1.97	1.91	1.66
Positive	5.36	6.35	4.96	5.38	5.51
Total (n)	3,208	3,071	3,043	3,607	12,930

FLUS/AUS: Follicular Lesion of Undetermined Significance/Atypia of Undetermined Significance. FN: Follicular Neoplasm

Table 2 summarizes the 4-year cyto-histologic correlation.

4 Years Cytology-Histology Patient Correlation

2008-2011	Cytology FNA Results						
Histology	Non-Diagnostic	Negative	FLUS/AUS	FN	Suspicious	Positive	Total
Malignant	23.80%	18.00%	56.80%	32.70%	91.00%	99.60%	52.60%
Neoplastic	10.40%	7.70%	15.00%	41.30%	2.40%	0.00%	11.70%
Non-neoplastic	65.90%	77.30%	28.20%	26.00%	6.60%	0.40%	35.70%
Total Surgery Patients #(%)	164 (13.4)	621 (6.5)	287 (43.2)	400 (43.2)	166 (77.6)	561 (79.2)	2,199 (17.0)

FLUS/AUS: Follicular Lesion of Undetermined Significance/Atypia of Undetermined Significance. FN: Follicular Neoplasm

The false positive rate for a malignant and suspicious diagnosis was 0.4% and 9.9%, respectively. The specificity of diagnosing malignant thyroid nodules was 97.7% whereas the specificity as a screening test for all neoplasms was 89.6%.

Conclusions: The BSRTC shows excellent specificity in diagnosing malignant nodules and in screening for neoplasms. Each diagnostic category conveys specific risks of malignancy, which offers guidance for clinical management. In addition, the frequency distribution of the individual diagnostic categories remained relatively stable over time.

426 Cytomorphology of Non-Small Cell Lung Carcinoma (NSCLC) with Anaplastic Lymphoma Kinase (ALK) Gene Rearrangement

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Background: ALK is a receptor tyrosine kinase demonstrating activating mutations in several malignancies including a subset (1-5%) of non-small cell lung carcinomas (NSCLC). The classic presentation is a relatively young patient with a minimal or absent smoking history, and lacking *EGFR* and *KRAS* mutations. While demonstrating resistance to both standard chemotherapy and anti-EGFR therapies, targeting ALK fusion products has demonstrated response rates above 50% in clinical studies. Prior work examining the histologic features of these tumors found a spectrum of findings, notably a solid / acinar pattern with signet-ring cells, as well as a mucinous cribriform pattern. Herein we present the first study to date describing the cytomorphology of NSCLC harboring ALK rearrangements.

Design: A retrospective database search was conducted to identify cytologic specimens of NSCLC demonstrating ALK rearrangement. Cytogenetic analysis was performed with fluorescence in situ hybridization (FISH) to evaluate ALK gene rearrangement. A total of 13 cases were identified. The following features were noted in each case: background, cytomorphology, and nuclear detail. Our control population consisted of ten cases of NSCLC not harboring ALK rearrangements, and was evaluated using the same cytomorphologic features as the study group.

Results: A total of 13 specimens from 9 patients were obtained. The average age was 50.4 years (range 28-68 yrs) with a male predominance (78%). Five patients never smoked, and four patients had a remote smoking history. All cases demonstrated moderate to poor differentiation with a predominance of single cells showing anisonucleosis and frequent intracytoplasmic neutrophils. The cells were frequently plasmacytoid with bi- and multinucleation, as well as numerous signet ring cells. The background showed necrosis and acute inflammation, as well as rare mucin. The control cases showed cells with smaller, less pleomorphic nuclei and smaller nucleoli with more clusters / tissue fragments. Intracytoplasmic neutrophils were present focally in one case. The background showed less necrosis with more mucin.

Conclusions: Several unique cytomorphologic features were consistently identified in the study population relative to the control population and include a prominence of single, markedly enlarged tumor cells with plasmacytoid features and anisonucleosis, as well as intracytoplasmic neutrophils. Larger studies are warranted to confirm our preliminary findings, as these features may help establish a more cost-effective means to select patients being tested for ALK mutational analysis.

427 Touch Preparations of Core Biopsies. Impact on Target Therapy Selection Eligibility

LC Tong, D Rudomina, M Friedlander, O Lin. Memorial Sloan-Kettering Cancer Center, New York, NY.

Background: Core biopsies (CB) have played an increased role in the management of different cancers and, sometimes even replaced fine needle aspiration specimens. These CB are often used to perform ancillary studies such as immunohistochemical and molecular studies. These ancillary studies are critical for selection of targeted therapy in certain organs and cancers. Although most of these studies can be performed on cytologic material, CB are often used instead due to protocol requirements. Many institutions have used touch preparations (TP) to evaluate the adequacy of CB at the time of procedure instead of the traditional frozen section. The evaluation of TP can provide rapid on site evaluation that a target lesion has been sampled, but the impact of TP on the adequacy of CB for diagnosis and ancillary studies for target therapy selection has not been addressed in a large series.

Design: CB specimens performed by interventional radiologists during a period of 6 months were included in this study. The CB were obtained from many different sites, including but not restricted to lung, kidney, lymph nodes, liver and soft tissue. A TP slide of the resulting core was prepared by the radiologist and subsequently air dried and stained with a modified Giemsa stain. The TP specimen was evaluated on site by a cytotechnologist to assess specimen adequacy. TP specimens were later evaluated by a cytopathologist, while CB were evaluated by a surgical pathologist. The diagnoses of the TPs and concurrent CB were compared to identify discrepancies.

Results: A total of 1221 CB were included in this series. A discrepancy between the CB and the TP was identified in 53 cases (4.3%) of cases. The reason for the discrepancy in 20 cases (1.6%) was the presence of neoplastic cells in the TP slides only, with no

diagnostic material identified in the CB. The discrepancy in the remaining 33(2.7%) cases was due to the presence of diagnostic neoplastic cells in the CB only with the TPs containing either only benign elements or too few cells to be considered diagnostic.
Conclusions: TP represents a convenient method to assess whether a target lesion has been sampled; however, the use of TPs might lead to depletion of neoplastic cells in the CB as most neoplastic cells may be removed and retained on the TP slide. The loss of neoplastic cells in the CB has the potential to hinder the evaluation of markers used for target therapy selection. Furthermore, TPs with low cellular yield may lead to false negative results in corresponding CB.

428 Endobronchial Ultrasound-Guided Transbronchial Needle Aspiration (EBUS-TBNA) Cytology: Review of High-Volume Institutional Experience with a Focus on Diagnostic Accuracy and *EGFR/KRAS* Molecular Testing

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Background: Only few large-scale studies have reviewed the performance of EBUS-TBNA cytology in the diagnosis and molecular analysis of lung carcinomas. We retrospectively reviewed our institutional experience with EBUS-TBNA samples, with a focus on cytologic/histologic correlation and sufficiency for *EGFR*/*KRAS* molecular testing.
Design: Cytologic/histologic correlation was performed for all EBUS-TBNAs obtained during a 1-year period (Jan – Dec 2009). In addition, results of routine testing for *EGFR* and *KRAS* mutations (by Sequenom and fragment analysis) on EBUS-TBNA samples, performed by request of clinicians, during 1.5-year period (Jan 2010 - June 2011) were reviewed, and 70 consecutive samples diagnosed as adenocarcinoma were scored for tumor cellularity to determine potential sufficiency for molecular testing in unselected samples. Based on the requirements in our molecular laboratory, sufficiency was defined as ≥10% tumor cell proportion and absolute cellularity of ≥200 cells.
Results: A total of 562 EBUS-TBNAs from 300 patients were performed in 2009. Cytology samples were classified as malignant or suspicious (n=231, 41%), atypical (n=1; 0.2%), negative (n=224; 40%) or non-diagnostic (n=106; 19%). Of 231 malignant diagnoses, a specific tumor type was determined in 191 (83%) cases. 123 of 562 (22%) EBUS-TBNAs had same-site histologic follow-up, revealing sensitivity and specificity for malignancy of 97% and 98%, respectively, and tumor typing accuracy of 100%. A total of 57 samples underwent routine molecular analysis, revealing 10 (18%) *EGFR* and 19 (33%) *KRAS* mutations. For 70 consecutive samples, 60 (86%) had sufficient material for molecular testing in the cell block (n=38) or smears (n=22) if cell block was unavailable or insufficient. The mean proportion of tumor cells (relative to benign cells) was 63% for cell blocks and 62% for smears.
Conclusions: Based on the review of our high-volume institutional experience with EBUS-TBNAs, we confirm a high sensitivity and specificity of this modality for the detection of malignancy. Furthermore, we find that EBUS-TBNA samples provide high accuracy of tumor type determination and yield sufficient material for molecular testing in the vast majority of cases.

429 Correlation of FISH with Atypical Urine Cytology and Its Progression to Malignancy

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Background: Fluorescent in situ hybridization (FISH) has been argued as a possible replacement for urine cytology to improve means of diagnosing new and recurrent urothelial carcinoma. In this study, we compiled the results of multicolor FISH tests performed on specimens diagnosed as "atypical urothelial cells" to determine the potential of FISH in identifying underlying malignancy when cytology specimens were diagnosed as atypical.
Design: From January 2008 to December 2010, all atypical urine cytology specimens at our institution were sent for FISH analysis to determine aneuploidy on chromosomes 3, 7, 17 or deletion on 9p21. We recorded and analyzed all completed FISH results to determine the sensitivity and specificity of these markers by correlating them with positive cytology and surgical pathology follow-up through June 2011.
Results: A total of 251 atypical urine cytology specimens were analyzed for FISH over three years and 22% (55/196) had positive FISH results. When comparing FISH results on atypical cytology with follow-up malignant diagnoses on cytology and surgical pathology specimens through June 2011, we found there were 23 true positives (TP), 32 false positives (FP), 177 true negatives (TN), and 19 false negatives (FN). Thus there was a sensitivity of 54.8% and a specificity of 84.7%. The positive predictive value (PPV) was 41.8% and the negative predictive value (NPV) was 90.3%.

Cytology/Histology Diagnosis Concurrence with FISH Results

	Specimen Positive	Specimen Negative
FISH Positive	23	32
FISH Negative	19	177

Furthermore, when comparing high-grade (HG) and low-grade carcinoma (LG) results, we found that of the true positives, 13 were HG and 6 were LG. There was also 1 renal cell carcinoma, and 3 positive cytology results with no histologic follow-up. Of the false negatives, there were 8 HG and 8 LG while 3 others showed positive cytology with no histologic follow-up.
Conclusions: Our FISH statistical results are similar to recently published studies that have evaluated the sensitivity and specificity for detecting malignancy in urinary specimens. The difference in our study is that we chose to focus exclusively on atypical urine cytology. While FISH results are able to detect true urothelial carcinoma in some cases, based on our data we do not believe it is a replacement for cytologic analysis. Rather, FISH may be used as an adjunct to urine cytology in certain situations.

430 Predictive Value of Pre-Operative Thyroid FNA BRAF Mutation Testing for Risk of Lymph Node Thyroid Carcinoma Metastases: A Single Institution Experience of 498 Cases

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Background: Thyroid fine needle aspiration (FNA) is standard in the evaluation of thyroid nodules for carcinoma. Recently, the utilization of reflex BRAF mutation adjunct testing has afforded cytology a diagnostic tool with prognostic implications. BRAF positivity is associated with compromised prognostic findings including: lymph node positivity, recurrence/reoperation risk, and radioiodine resistance. In this study we evaluate the predictive value of pre-operative thyroid FNA BRAF mutation testing in regard to finding cervical lymph node metastases.
Design: We reviewed 498 consecutive thyroid FNAs performed on 447 patients between 2009 and 2012, the period in which reflex BRAF mutation testing was available at our institution. Average patient age was 53 years, with men and women represented equally. Reflex BRAF testing was initiated on diagnoses of atypia (of undetermined significance), and those suspicious/definitive for malignancy.
Results: The results showed: 173/498 (35%) cases where BRAF was mutated, 294/498 (59%) where BRAF was wild type (WT), and 31/498 (6%) where BRAF was inconclusive. 283 of 498 cases had a thyroidectomy with or without a lymphadenectomy (LN); representing 118 BRAF mutated, 147 BRAF WT, 18 BRAF inconclusive cases. 77 of 283 cases had positive lymph nodes as follows:

Lymph Node Positivity as Predicted by BRAF

	Node Status			
BRAF status	Cases (N)	Central Nodes, Level VI (+)	Lateral Nodes, Levels II-V (+)	Total Nodes (+)
BRAF mutated	118	26	13	39
BRAF WT	147	17	11	28
BRAF	18	8	2	10
Total	283	51	26	77

LN: Lymphadenectomy

Sensitivity was 58%; specificity was 100%, while positive and negative predictive values were l00% and 88%, respectively. The Likelihood Ratio was 35.4.
Conclusions: The utilization of reflex BRAF molecular testing on thyroid FNA specimens has prognostic utility in predicting lymph node metastases. Based on these findings, prophylactic central lymphadenectomy may be considered at the time of thyroidectomy in patients with BRAF mutations diagnosed on pre-op FNA.

431 Atypia in Follicular Neoplasm: Making a Case for Follicular Variant of Papillary Thyroid Carcinoma

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Background: The Bethesda thyroid classification describes follicular neoplasm (FN) as a cellular lesion showing microfollicular architecture with scant or absent colloid. Fine-needle aspiration (FNA) diagnosis of FN is a screening test that does not differentiate between a benign and malignant tumor. The majority of thyroid nodules (up to 80%) diagnosed as FN are benign upon histologic examination. This study is designed to determine the predictive value of cytologic diagnosis in a subset of FN and offer a practical guide for thyroid physicians by identifying significant risk factors for malignancy based on cytologic atypia.
Design: Based on a retrospective review of cytologic diagnosis between January 2008 and December 2011, all thyroid FNA cases with the diagnosis of FN were reviewed. A subset with cytologic atypia – some features suggestive but not diagnostic for Papillary thyroid carcinoma follicular variant (FVPTC) – was identified. The PPV of the cytologic interpretation of FN with atypia for neoplasia (including adenoma and carcinoma) and that for malignancy were calculated.
Results: A total of 38 cases of thyroid FNA (29 females and 9 males) with the cytologic diagnosis of FN with atypia (and with surgical follow-up) were identified (representing 12% of the total number of cases diagnosed as FN with surgical follow-up over this time period). All patients had undergone either lobectomy with completion thyroidectomy or total thyroidectomy. The 38 FNA samples resulted in the following distribution of final histological diagnosis: Neoplastic – 30/38 (out of which 26 were malignant), Benign – 8/38. The positive predictive values for neoplasia and malignancy were 78% and 68% respectively. The malignant cases were predominantly FVPTC (19/26). Others included classic PTC (5/26) and follicular carcinoma (2/26).
Conclusions: The reported incidence of malignancy in FN is 10%-30%. FN with subtle atypical features has a much higher rate of malignancy (68%). The main diagnostic challenge is to differentiate FVPTC from other follicular lesions. Subclassifying FN based on presence of atypia has implications for management. This subset of patients will benefit from a more aggressive follow-up including immediate referral for lobectomy.

432 Utility of Islet 1, PAX8, CDX2 and TTF1 in Fine Needle Aspiration Workup of Metastatic Neuroendocrine Tumors

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Background: Determining the origin of a metastatic neuroendocrine tumor (NET) can be challenging since primary NET from various sites share similar cytologic features. CDX2 and TTF1 have been found helpful identifying well-differentiated primary NET of gastrointestinal (GI) and pulmonary origin, respectively. Islet1 and PAX8 are useful markers for pancreatic endocrine neoplasia (PEN). We evaluated a panel of markers to determine the origin of metastatic NET on fine needle aspiration (FNA) cytology.
Design: FNA of 20 cases of primary NET (16 pancreatic, 5 pulmonary, 1 ileal, 1 duodenal, 1 colonic, and 1 gastric) and 31 metastatic NET (13 pancreatic, 10 pulmonary, 6 ileal, 1 duodenal, and 1 rectal) were evaluated. Cell blocks were

immunohistochemically (IHC) stained for PAX8, Islet 1, TTF1 and CDX2. Nuclear staining for all antibodies was independently scored by two investigators as negative (<5% positivity) or positive (>5% moderate strong positivity).
Results: Islet 1 and PAX8 were positive in 13/16 (81.2%) and 14/16 (87.5%), respectively, of primary PEN. 10/13 cases (76.9%) of metastatic PEN were positive for either Islet1 or PAX8. Coexpression of both was present in 12/16 (75.0%) and 9/13 (69.2%) primary and metastatic PEN, respectively. No cases of primary or metastatic ileal NET were positive Islet1 or PAX8. PAX8 was positive in 1/1 primary gastric, 1/1 primary duodenal and 1/5 primary pulmonary NET. Islet 1 was expressed in 1/1 primary duodenal and 1/5 primary pulmonary NET. CDX2 expression was present in all cases of primary duodenal, ileal, colonic and rectal NET and 5/6 (83.3%) metastatic ileal NET. 1/16 (6.25%) primary and 2/13 (15.4%) metastatic PEN were positive for CDX2. TTF1 was positive in all cases of primary and metastatic pulmonary NET.
Conclusions: 1.Islet 1 and PAX8 were specific and sensitive for metastatic PEN 2.Although there was no significant difference between Islet1 and PAX8 in sensitivities and specificities for primary and metastatic PEN, 18.7% primary and 15.3% metastatic PEN showed discordant staining, suggesting that the stains complement each other as part of IHC panel for the FNA work-up of PEN 3.Islet 1 and PAX8 expression was negative in primary and metastatic ileal NET compared with high expression in primary and metastatic PEN, suggesting that Islet 1 and PAX8 can be useful in the FNA cytology work-up of ileal versus pancreatic metastatic NET 4.CDX2 and TTF1 were specific for primary and metastatic GI and pulmonary NET 5. We recommend Islet 1, PAX8 in addition to TTF1, and CDX2 for the work-up of metastatic NET of unknown primary in FNA cytology.

433 Utility of Islet-1, CD99, PAX8 and ß-Catenin in Fine Needle Aspiration Workup of Non-Ductal Pancreatic Neoplasms

C Velosa, J Nunez, JF Silverman. Allegheny General Hospital, Pittsburgh, PA.
Background: Endoscopic ultrasound guided fine-needle aspiration (FNA) is a useful method for the diagnosis of pancreatic neoplasms, including pancreatic endocrine neoplasms (PEN), acinar cell carcinoma (ACC), solid-pseudopapillary neoplasm (SPN) and ductal adenocarcinoma (DA). However, the cytologic diagnosis of the non-ductal neoplasms can occasionally be challenging due to overlapping diagnostic features. We evaluated a panel of antibodies to determine which markers could provide the best immunophenotypic characterization for problematic cases of PEN, ACC and SPN on FNA cytology.
Design: 37 cases of FNA of pancreatic neoplasia were selected. Cell blocks from 16 PEN, 6 SPN and 3 ACC and 12 DA were immunohistochemically (IHC) stained for PAX8, CD99, b-catenin, CD10, Islet 1, DOG1, synaptophysin (Sy), chromogranin (Ch), a1-antitrypsin (AT), a1-chymotrypsin (ACT). The slides were independently scored by two investigators as negative (<5% positivity) or positive (>5% moderate strong positivity).
Results: PEN showed strong nuclear staining for PAX 8 in 14/16 (87.5%) cases and Islet 1 in 13/16 (81.2%) cases. Membranous staining for CD99 was present in 12/16 (75.0%) of PEN. All cases of PEN were positive for Sy and Ch and negative for CD10, DOG1, b-catenin, AT and ACT. Strong nuclear staining for b-catenin was present in all cases of SPN. 5/6 (83.3%) of SPN showed perinuclear dot staining for CD99 and 4/6 (66.6 %) cases were positive for CD10. 2/6 (33.0%) of SPN showed coexpression of Sy and Ch and PAX8 was positive in 2/6 (33.3%) of SPN. 1 SPN showed coexpression of the three markers and 1/6 (16.6%) SPN showed staining for AT. There was no expression of DOG-1 in SPN. All cases of ACC showed positive staining for AT and ACT. 1 case of ACC coexpressed CD10, Sy and Ch. DOG-1 was the only positive staining in DA, in 2/12 (16.6%) cases.
Conclusions: 1. SPN, PEN and ACC showed overlapping of immunophenotypic patterns, especially for neuroendocrine markers.
2. In differentiating SPN from PEN, β-catenin and CD99 (perinuclear dot staining) expression was highly specific (100/100%, respectively) and sensitive (100%/ 83%, respectively) for SPN.
3. Islet 1 was the most specific (100%) marker for PEN. Although PAX-8 was highly sensitive (87.5%) for PEN, it was less specific (90%) than Islet-1, since it was also present in 33% of SPN.
4. AT expression was highly sensitive (100%) for ACC, but less specific (97%) since it also stained 16% of SPN, but no PEN.
5. We recommend β-catenin, CD99, PAX8 and Islet 1 to be included in the IHC panel for the FNA cytology work-up of non-ductal pancreatic neoplasms.

434 The Combination of ProEx C and uCyt Tests Improve Detection of Urothelial Carcinoma in Atypical Urine Samples

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Background: Detection of urothelial cell carcinoma (UCC) by urine cytology can be challenging, especially for low grade tumors, and adjunctive markers, such as ImmunoCyt (uCyt), are useful. Recently, we found that ProEx C may be an additional molecular marker to improve detection of UCC. ProEx C, originally developed for assessing dysplasia in gynecologic specimens, is an assay targeting expression of topoisomerase II-α & minichromosome maintenance protein-2. The current study evaluated the utility of ProEx C combined with uCyt as adjunct markers in urine cytology.
Design: A retrospective study consisting of a total of 71 patients with the diagnosis of urothelial cell atypia was performed, all of which had prior uCyt tested. Archived ThinPrep slides were restained with ProEx C immunoassay. ProEx C was recorded as positive when nuclear staining was seen in atypical urothelial cells. uCyt was considered positive if ≥5 cells were seen to fluoresce green or red. All cases had follow-up surgical biopsy (13 negative, 18 low grade UCC, 37 high grade UCC).
Results: When ProEx C and uCyt were performed individually, each had an 84% positive rate for high grade UCC. Although ProEx C had a slightly low positive rate in low grade UCC compared to uCyt (72% versus 83%), the positive rate for ProEx C vs uCyt in the negative follow-up group was also slightly low (i.e. false positive rate, 38% versus 54%). The combination of the two tests in an 'either/or' setting detected 17 out of 18 (94%) low grade tumors and 34 of 37 (92%) high grade tumors (p=0.06 and p=0.02, respectively, compared to negative group); however, 8 of 13 (62%) negative cases were positive for ProEx C or uCyt.

Results of ProEx C, uCyt, and ProEx C/uCyt with Histologic Correlation

		Histology			
TEST	Staining Results	Negative, n=13 (%)	Low Grade, n=18 (%)	High Grade, n=37 (%)	Combined Low & High Grade, n=55 (%)
ProEx C	+	5 (38)	13 (72)*	31 (84)**	44 (80)**
	-	8 (62)	5 (28)	6 (16)	11 (20)
uCyt	+	7 (54)	15 (83)	31 (84)	46 (84)**
	-	6 (46)	3 (17)	6 (16)	9 (16)
ProEx C/uCyt	+	8 (62)	17 (94)*	34 (92)**	51 (93)**
	-	5 (38)	1 (6)	3 (8)	4 (7)

*two-tailed p value <0.1, **p value <0.05, in comparison to negative group
Conclusions: The combination of the two tests yielded high sensitivity for detecting not only high grade tumors, but low grade UCC as well. The two tests may be used together to improve detection of UCC in atypical urine samples.

435 Variable Impact of Qualifying Language on Clinical Management of Atypical Thyroid Fine Needle Aspirates

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Background: The Bethesda System for Reporting Thyroid Cytopathology (TBS) category of atypia of undetermined significance/follicular lesion of undetermined significance (AUS/FLUS), is used to classify a variety of mild cytologic abnormalities in thyroid FNAs. Modifying terminology is often added in thyroid FNA reports for descriptive and risk assessment purposes, but it is not known whether specific phrases affect patient management. To answer this question, we correlated rates of excision and re-biopsy among patients with AUS/FLUS diagnoses from Baptist Hospital, Miami, FL (BH) and Brigham and Women's Hospital, Boston, MA (BWH) with the language used in thyroid FNA reports.
Design: We identified thyroid nodules with an initial FNA diagnosis of AUS/FLUS at both institutions. In total, we studied 314 FNAs from BH including 251 females and 63 males with a median age of 53 (21-79), and 300 FNAs from BWH including 241 females and 59 males with a median age of 66 (range 10-85). Pathology reports from all cases were evaluated for common descriptive phrases and language known to be associated with differences in risk of malignancy.
Results: Significantly more patients underwent excision at BH than at BWH (39% vs 8%, p<0.001), and significantly fewer had a repeat biopsy (15% vs 92%, p <0.001). AUS /FLUS qualifiers shown to be associated with different risks of malignancy (rule out papillary carcinoma, atypia NOS, cytologic atypia, architectural atypia) had no impact on rates of re-biopsy or excision (p >0.05 for all). A recommendation for repeat biopsy increased the rate of re-biopsy at BH (17% vs 4.8%, p = 0.02), but not at BWH (93% vs 91%, p =0.67). Use of the phrases "favor benign" or "likely benign" resulted in a decreased rate of excision at BH (14% vs 43 %; p=0.05), but were not used in BWH reports. Phrases suggesting uncertainty of malignancy, inadequate sampling, or any other phrase containing the word "benign" had no effect on treatment at BH or BWH (p>0.05 for all).
Conclusions: Management of patients with a diagnosis of AUS/FLUS in a thyroid FNA varies significantly. In a setting that closely adheres to proposed management guidelines of TBS, descriptive phrases do not modify clinical management of patients with an initial AUS/FLUS diagnosis. However, in a setting where most patients do not undergo repeat biopsy, the phrases "favor benign," "likely benign," and "recommend repeat biopsy" are more likely to affect patient management than descriptive qualifiers associated with different risks of malignancy.

436 p40 (ΔNp63): A Highly Sensitive and Specific Immunohistochemical (IHC) Marker for Diagnosing Pulmonary Squamous Cell Carcinomas (SQCC) in Fine Needle Aspirates

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Background: The treatment of pulmonary non-small cell carcinoma has become sharply divided between the therapeutic options now devoted to squamous cell carcinoma (SQCC) and those of adenocarcinoma (ADC). Due to the increasing ability to make a diagnosis on minimal tissue, ancillary techniques such as immunohistochemistry (IHC) are needed and must be highly sensitive and specific. The popular squamous cell IHC marker p63 has demonstrated cross-reactivity with a subset of pulmonary adenocarcinomas and lymphomas, causing it to be less specific than originally thought. The IHC marker p40 (ΔNp63) is a truncated isoform of p63 that is a promising IHC marker for SQCC. In this study we have studied and compared its utility with p63 and cytokeratin 5 on fine needle aspiration (FNA) cell blocks (CB).
Design: Thirty cases of pulmonary SQCC and thirty cases of pulmonary ADC with CB were selected. IHC for p40 (ΔNp63), p63, and cytokeratin 5 were performed on all paraffin-embedded CB serial sections.

Results:

Marker expression in pulmonary squamous cell carcinoma and adenocarcinoma

Squamous cell carcinoma				Adenocarcinoma			
Marker	Positive	Negative	Percent Positive	Marker	Positive	Negative	Percent Positive
p40	30/30	0/30	100%	p40	0/30	30/30	0%
p63	29/30	1/30	97%	p63	6/30	24/30	20%
CK5	29/30	1/30	97%	CK5	0/30	30/30	0%

Marker expression specificity and sensitivity in non-small cell carcinoma

Marker	Specificity	Sensitivity	PPV	NPV
p40	100%	100%	100%	100%
p63	80%	97%	83%	96%
CK5	100%	97%	100%	97%

PPV: Positive Predictive Value; NPV: Negative Predictive Value

All cases (n=30) of squamous cell carcinoma stained positive for p40 (ΔNp63). All cases of pulmonary adenocarcinoma were negative for both p40 (ΔNp63) and cytokeratin 5. Six cases (20%) of pulmonary adenocarcinoma demonstrated nuclear staining in at least 10% of malignant cells.

Conclusions: Differentiating between pulmonary SQCC and pulmonary ADC in FNA CB can sometimes be challenging. In this regard, our data support p40 (ΔNp63) to be more sensitive and specific and to possess a greater positive and negative predictive value for SQCC in comparison to p63. This study also documents that p40 (ΔNp63) does not stain ADC, which p63 does in 20% of cases. We also found that p40 (ΔNp63) shows a greater sensitivity and negative predictive value when compared to cytokeratin 5. In paucicellular CB the increased indices p40 (ΔNp63) provides may be extremely helpful in confirming the diagnosis of SQCC which may have significant therapeutic implications.

437 Comparison of ER, PR and HER2 Expression between Breast Carcinoma Fine Needle Aspiration Samples and Corresponding Surgical Pathology Specimens – A Large Retrospective Study

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Background: Determination of estrogen receptors (ER), progesterone receptors (PR) and HER2 is standard in breast carcinomas. ER, PR and HER2 are routinely evaluated on fine needle aspiration (FNA) cell blocks (CB) and tissue sections using immunohistochemistry (IHC). Whether FNA CB are reliable for these biomarkers remains controversial. The objective of this study is to determine concordance between these biomarkers on cell blocks versus tissue blocks.

Design: A large retrospective study was performed in 150 consecutive cases from 2002 to 2009. FNA CB and corresponding tissue blocks of invasive breast carcinoma were identified. We compared ER, PR and HER2 immunohistochemical expression in formalin-fixed FNA CB and subsequent formalin-fixed tissue blocks from the same patient. Tissue blocks were formalin fixed per ASCO/CAP guidelines for HER2 (IHC). Fluorescence in situ hybridization, performed on a subset, was included in the analysis.

Results: We found agreement in 98% of cases with positive ER expression and 100% agreement with negative ER expression, using findings on tissue blocks as gold standard. PR correlation was slightly lower (96%) than ER for positive expression but 100% agreement with negative expression. HER 2 testing demonstrated > 95% agreement between CB and tissue block samples.

Conclusions: This study is the largest study to date, to our knowledge, correlating ER, PR and HER2 determination on cell block and subsequent tissue samples. In addition, this study is different from most previous correlation studies in that cell block samples were fixed directly in formalin. Based on the excellent concordance found we conclude that FNA CB samples are reliable for the assessment of these biomarkers.

438 Atypia of Undetermined Significance (AUS) in Thyroid FNA. Malignancy Rate and Value of Substratification

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Background: The Bethesda System for reporting thyroid cytopathology (TBSRTC) includes a category of Atypia of Undetermined Significance (AUS). The AUS category has an implied risk of malignancy between 5-15%according to TBSRTC, although several studies have shown a higher rate of malignancy, ranging from 20 to 40 %. Our objective was to analyze the rate of malignancy in thyroidectomies that had a previous diagnosis of AUS at a comprehensive cancer center, where the rate of AUS diagnosis is 8%, close to the recommended rate by TBSRTC. We also proposed to evaluate the role of substratifying the diagnosis of AUS.

Design: Patients with a thyroid FNA diagnosis of AUS who underwent thyroidectomy between 2007-2011 were selected for this study.The diagnosis was further stratified into 5 categories as following: AUS, NOS; AUS favors benign; AUS cannot rule out papillary thyroid carcinoma (PTC); AUS cannot rule out Hurthle cell neoplasm; and AUS cannot rule out follicular neoplasm. The cytologic diagnoses were correlated with findings in the thyroidectomy specimens to determine the benign (goiter and lymphocytic thyroiditis), neoplastic (including adenomas) and malignancy rate.

Results: The findings in 139 patients who underwent thyroidectomies following a diagnosis of AUS were analyzed. The rate of malignancy in the resected nodules matching the AUS diagnosis was 37% overall, however an additional 23% of patients also had an incidental microcarcinoma not sampled by FNA. Upon stratification of the AUS category as proposed above, the malignancy rate was significantly higher ($p<0.001$) if a specific neoplasm could not be ruled out when compared to the AUS, NOS or AUS, favor benign. A summary of the malignancy rate in each AUS subcategory is listed in table 1.

Table 1: Histologic correlation in each AUS subcategory

FNA/thyroidectomy (number of cases)	Benign (%)	Neoplasm (%)	Malignant (%)
AUS only,NOS (65)	60	40	25
AUS, favor benign (13)	77	23	8
AUS,cannot r/o PTC (13)	23	77	54
AUS, cannot r/o Hurthle cell neoplasm (15)	26	74	47
AUS, cannot r/o follicular neoplasm (33)	18	82	59

r/o: rule out; PTC: papillary thyroid carcinoma

Conclusions: The rate of malignancy in the AUS category is higher than the proposed in the TBSRTC. If a specific lesion cannot be ruled out, surgery should be considered due to the high risk of the presence of a neoplasm. Our results suggest that AUS substratification allows a better selection of patients for surgery.

439 Evaluating Diagnostic Concordance of 3-D Virtual Gynecologic Imaging vs. Light Microscopy: Follow-Up Study

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Background: Virtual microscopy (VM) in cytology has been limited by the inability to focus through 3-dimensional cell clusters with single focal plane (2-dimensional) imaging. We previously established an optimal scanning interval of one micron, and further investigated the optimal number of scanning focal plane levels (3). The goal of this study was to confirm if 3 focal plane levels at 1 micron is comparable to light microscopy (LM) for gynecologic specimens.

Design: Two sets of 96 SurePath™ slides (192 slides total) were scanned at 40X magnification with 3 focal planes at one micron using an iScanCoreo Au scanner (Ventana, AZ, USA), generating two sets of virtual images. The four subsequent sets (two glass, two virtual – 384 slides total) were evaluated by two cytopathologists, two pathology residents, and one cytotechnologist. Bioimagene's Image Viewer (version: 3.0.0.0-RC8) and conventional LM were used to diagnose pre-annotated cells using selected standard squamous epithelial Bethesda diagnostic categories. The slides were evaluated in sets which were presented in variable order.

Results: The intra- and interobserver reliability were evaluated using Kappa statistics. Eight cases (4.2%) of VM slides were designated (by at least one observer) "unable to diagnose" secondary to poor image quality. These cases were included in the final statistics. The overall intraobserver diagnostic concordance between glass and virtual slides was very high (87-97%). Interobserver agreement (in which all five observers gave the same diagnosis) was evaluated, and for all cases was found to be higher for LM (94%) compared to VM (82%). In positive cases (low-grade or high-grade squamous intraepithelial lesion), the interobserver agreement was also higher for LM (76%), and lower for VM (55%). The overall concordance between all observers (LM and VM) was 76%. Concordance of LM and VM with established biopsy diagnosis was lower.

Conclusions: Excellent intraobserver concordance between LM and VM was established. While interobserver agreement was higher for LM, it was still very good using VM. An additional study incorporating more stringent quality control of virtual images may be required to fully establish biopsy:LM:VM diagnostic concordance and method equivalence.

440 The Clinical Utility of HPV DNA, mRNA and p16INK4A/Ki-67 as Triage Tools for Low Grade Cervical Lesions LSIL and ASCUS

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Background: There remains considerable challenges over the management of low grade abnormalities, due to the high prevalence of transient HPV infections in low-grade disease HPV DNA triage is limited. The use of HPV E6/E7 mRNA detection and biomarkers such as p16INK4A and Ki-67 has potential to identify clinically significant infections improving diagnostic specificity. The aim of this study is to investigate the utility of these markers for detecting women at risk of developing high grade disease presenting at colposcopy with LSIL/ASCUS.

Design: Cervical smears for HPV testing and immunocytochemical analysis were collected from 1079 women presenting with LSIL/ASCUS at their first visit to colposcopy at the National Maternity Hospital, Dublin. HPV DNA was detected by Hybrid Capture II (Qiagen, UK), HPV E6/E7 mRNA expression by the PreTect™ HPV Proofer (NorChip AS, Norway) and p16INK4A/Ki-67 expression was assessed using CINtec PLUS (Roche).

Results: HPV DNA and mRNA testing has been performed on 1079 patient samples, a subset of 430 cases have been tested for p16INK4A/Ki-67 dual expression. Over all HPV DNA testing provided the highest sensitivity, 93% (95% CI 0.9156-0.9455), for detection of CIN 2+. However, HPV mRNA and p16INK4A/Ki-67 co-expression provided enhanced specificity 70% (95% CI 0.7277-0.8495) and 84% (95% CI 0.8232-0.859). Analysis is currently underway to investigate combined HPV and immunocytochemical testing for optimal clinical sensitivity and specificity.

Conclusions: This offers prospective evidence that HPV testing in the management of women presenting with low grade abnormalities could be useful in detecting those at risk of developing high grade disease. This study is carried out under CERVIVA the Irish Cervical Screening Research Consortium funded by the Health Research Board and is supported by The Irish Cancer Society.

441 Three Dimensional Cell Groups with Disordered Nuclei and Cellular Discohesion Are Associated with High Sensitivity and Specificity in the Cystoscopic Urine Diagnosis of Low Grade Urothelial Neoplasia

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Background: A review of cystoscopic urine obtained before the resection of low grade urothelial carcinoma (LGUC) frequently revealed the presence of three dimensional cell groups with disordered nuclei and cellular discohesion (3D-DD). The purpose of this study is to determine the clinical significance of this finding.

Design: 936 consecutive cystoscopic urine specimens were reviewed and divided into 5 groups. Groups 1 (80 specimens) and 2 (20 specimens) included patients with biopsy-proven LGUC collected during follow-up periods of 0-6 months and 6 months to 2 years, respectively. Group 3 (300 specimens) included urine from patients with hematuria and insignificant cystoscopic findings. Group 4 (6 specimens) consisted of patients with a history of urinary stones. Group 5 (530 specimens) included patients with a history of LGUC but no evidence of cystoscopic recurrence.

Results: Specimens with scant cellularity accounted for 20% of the specimens in Group 1, likely associated with technical problems from collection. Tumors with increased cell adhesion or a small exophytic component may also contribute to poor specimen collection. 3D-DD was present in most cases with mild cytologic atypia, and in all cases with papillary stromal fragments. 3D-DD was often associated with increased cellularity and an abundance of single cells. Assuming a 20% inadequate sampling rate over the five specimen groups, 3D-DD was associated with a 72% sensitivity, 96% specificity, 90% positive predictive value (PPV) and 89% negative predictive value (NPV). Two or 3-dimensional cell groups with ordered nuclei and/or cellular non-discohesion were often associated with Groups 3 and 4. Interestingly, 3D-DD was present in a significant number of cases that showed initial concurrent negative findings, but were associated with LGUC in follow-up cystoscopies. In both instances (negative cystoscopies or LGUC), 3D-DD was morphologically similar. Also, 3D-DD was present in 8% of Group 5 specimens, likely representing a possible LGUC or urothelial dysplasia not cystoscopically detectable and with low neoplastic progression.

Conclusions: Our study demonstrated the high specificity and sensitivity of 3D-DD for LGUC. These findings are consistent with the decreased cell adhesion and disordered nuclear arrangement of low grade urothelial neoplasia. Future studies with larger case numbers and longer follow-up periods are necessary to confirm our observations.

442 An Institutional Quality Assurance Study To Identify Factors Influencing Rates of Thyroid Bethesda System Categories among Cytopathologists

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Background: The Bethesda System for Reporting Thyroid Cytopathology (TBS) recommends a six category scheme for thyroid FNAs. Importantly, the new Atypia of Undetermined Significance/Follicular Lesion of Undetermined Significance (AUS/FLUS) category was introduced in 2008. We compared the rates of each TBS category and studied factors that may influence the rate of AUS/FLUS among 6 board-certified cytopathologists at our institution with lengths of practice ranging from 2-25 years.

Design: We identified all thyroid FNAs (n=927) from January 2010-September 2012 using CoPath. We then searched for corresponding surgical excisions to determine malignancy rates. Rates of TBS category and malignancy rates were calculated for each cytopathologist. We then dichotomized cytopathologists into those who began practice prior to the introduction of AUS/FLUS (pre-TBS) and those who began after (post-TBS). We compared the use of AUS/FLUS between pre- and post-TBS using the chi-square test. To assess if the malignancy rate of AUS/FLUS differed among the two groups, we computed Fisher's exact test.

Results: The overall laboratory rates were 9.7% unsatisfactory (U), 63.6% benign (B), 14.2% AUS/FLUS, 5.6% follicular neoplasm/suspicious for follicular neoplasm (FN/SFN), 2.9% suspicious for malignancy (SM) and 4.0% malignant (M). Individual pathologist rates ranged from 6.5-11.5% U, 56.9-75.4% B, 4.2-23.9% AUS/FLUS, 2.6-10.2% FN/SFN, 1.5-3.7% SM and 1.7-7.7% M. There were significant differences between pathologists in use of TBS categories ($p = 0.0005$). Only 1 pathologist classified <7% of samples as AUS/FLUS. There was a significantly lower rate of AUS/FLUS among pathologists who began practice before TBS (pre-TBS rate 10.3%, post-TBS rate 17.4%, $p=0.0016$). We identified 173 cases with follow-up histology (18.7%). Omitting unsampled incidental papillary microcarcinomas, overall laboratory malignancy rates by TBS category were 0% U, 6.25% B, 10.9% AUS/FLUS, 35.1% FN/SFN, 75.0% SM, 100.0% M. Malignancy rates for the AUS/FLUS category were 20.0% for pre-TBS pathologists vs. 5.7% for post-TBS ($p=0.1755$).

Conclusions: Board certified pathologists at our institution show significant differences in their use of TBS categories. Pathologists who began practice post-TBS had a higher rate of use of the AUS/FLUS category than pre-TBS pathologists, but differences in malignancy rates for these two groups were not statistically different.

443 Cellient™ Automated Cell Block System Is Useful in the Differential Diagnosis of Atypical Glandular Cells in Pap Tests

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Background: Atypical glandular cells (AGC) is an important diagnosis in gynecological cytology. Approximately 10 to 39% of AGC patients will be found to have high grade squamous lesions, glandular dysplasia, or cancer on follow up. The Cellient™ Automated Cell Block System allows efficient and rapid processing of micro-sized cytology samples for histologic sectioning. In this study, we investigate whether Cellient cell blocks could help to distinguish AGC from other cervical lesions on the cytologic Pap test.

Design: Between January 2010 and March 2012 in a large academic medical center, Cellient cell blocks were prepared on 71 cases for which AGC was considered based on the Pap test. Cellient utilizes a flow-through embedding technique and then automatically positions cell samples at a defined plane in paraffin blocks. H&E stained slides from the cell blocks were analyzed.

Results: Fifty five of 71 cases were given a diagnosis of AGC on the Pap smears whereas 16 cases were diagnosed as other than AGC on the Pap smears but were finally signed out by pathologists as AGC. Of the 55 cases, 26 (47%) were signed out by pathologists after reviewing the cell block slides as negative for AGC: diagnoses on the 26 cases were "questionable reactive", n=18; atypical squamous cell of undetermined significance (ASCUS), n=5; low-grade squamous intraepithelial lesion (LSIL), n=3. Eighteen of 55 (33%) cases were signed out as AGC (endocervical origin, n=8; endometrial origin, n=6; NOS, n=4). Importantly, 11 of the 55 (20 %) cases referred to pathologists as AGC were changed to other significant diagnoses including endocervical adenocarcinoma in situ, n=1; invasive carcinomas, n=4; atypical squamous cells, cannot exclude high-grade squamous intraepithelial lesion (ASC-H), n=3 and high-grade squamous intraepithelial lesion (HSIL), n=3. Sixteen of 71 cases referred to pathologists with a cytotechnologist diagnosis other than AGC ("questionable reactive", n=9; ASCUS, n=3; ASC-H, n=1; HSIL, n=1; "positive for malignant cells", n=2) were diagnosed as AGC (endocervical origin, n=9; endometrial origin, n=5; NOS, n=1; favor adenocarcinoma, n=1) after cell blocks were examined.

Conclusions: The Cellient™ Automated Cell Block System is a useful technique in the differential diagnosis of AGC. Cell blocks help rule out AGC so that unnecessary colposcopic evaluation or biopsies can be avoided. Cell blocks also help to confirm AGC diagnosis on equivocal smears.

444 Evidence That Judicious Use of a Cellient™ Cell Block and p16/Ki67 Immunohistochemistry (IHC) in Pap Tests Improves the Prediction of Biopsy Findings of CIN2 or Worse

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Background: P16 and Ki67 stains are increasingly used in cervical biopsies. Little data is available for use of p16 and Ki67 stains in cell blocks of Pap tests.

Design: 56 Pap tests between January 2010 and March 2012 with equivocal diagnoses on Pap smear were examined with H&E stained Cellient™ cell blocks and p16 and/or Ki67 immunohistochemistry (IHC). Cellient utilizes a flow-through embedding technique and automatically positions cells at a defined plane in paraffin to maximize cell yield. The final pathologist diagnoses based on review of the cell block and IHC findings were compared to the cytotechnologist diagnoses based on the Pap test alone. The diagnoses based on the Pap test alone and IHC slides were confirmed in a subset of cases. Two-tailed chi square test was used for statistical analysis.

Results: The cytotechnologist Pap test diagnoses on the 56 cases are shown in Table 1.

Table 1. Cytotechnologists diagnoses on pap test vs. cell block with IHC.

Cytotechnologist		Pathologist diagnoses integrating cell block with p16/Ki67 IHC					
Pap smear	Sub-total	NILM	ASCUS/LSIL	LSIL-H/ASC-H	AGC	HSIL	MALIGNANT
Reactive	13	2	4	3	3	1	
ASCUS/LSIL	15	2	5	4		3	1
LSIL-H/ASC-H	2			2			
AGC	18	4	3	1	6	2	2
HSIL	8			4		4	
Total	56	8	12	14	9	10	3

Diagnoses were changed following pathologist review of cell blocks and IHC slides in 37 cases (66%) whereas diagnoses were unchanged in 19 cases. Of the 37 altered diagnoses, 23 were upgraded and 14 were downgraded. Twenty seven of the 37 cases have follow-up biopsies or Pap tests, of which cervical intraepithelial neoplasia 2 (CIN 2) or worse were confirmed in 39% (7/18) upgraded cases versus 0% (0/9) downgraded cases ($P=0.03$) (Table 2). On selected review of cases, IHC stains were essential for both upgrading and downgrading.

Table 2. Follow-up on upgraded and downgraded Pap tests.

	Diagnoses on follow up		
	<CIN2	>=CIN2	Total
Upgraded	11	7	18
Downgraded	9	0	9
Total	20	7	27

<CIN2: Negative for malignancy, CIN1; >=CIN2: CIN2, CIN3, carcinoma in situ, adenocarcinomas.

Conclusions: Judicious addition of a Cellient cell block and p16/Ki67 IHC improves the prediction of biopsy findings of CIN2 or worse. Cell block and IHC may be cost-effective by obviating the need for colposcopy, or allowing patients to proceed directly to a diagnostic excisional procedure (e.g., LEEP) if a histologic diagnosis can be provided on the Pap test sample. A proposal for uniform criteria for scoring these immunostains on cell blocks of pap tests is presented.

445 Histologic Correlation of Fine Needle Aspirations of 101 Hurthle Cell Lesions

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Background: Hurthle cell lesions continue to be a confounding entity for cytopathologists where there are no definitive criteria to decisively distinguish Hurthle cell carcinoma from adenoma or adenomatoid nodules on cytology. Despite the limitations, this study aims to identify the common causes of discrepant results when a diagnosis of FLUS/AUS (Hurthle cell type), Suspicious for Hurthle cell neoplasm, or Hurthle cell Neoplasm is rendered by the cytopathologist.

Design: A total of 197 ultrasound guided-fine needle aspirations were identified from the archives of NYU Langone Medical Center and Bellevue Hospital Center during 2007 through 2012, consisting of consult cases and on-site ultrasound guided aspirations performed by NYU cytopathologists. 101 cases (51%) had surgical follow-up with either partial or total thyroidectomy resections. Diagnoses rendered before 2009 were converted to the current Bethesda terminology. Diagnoses of "Hurthle cell Nodules" were classified as "Suspicious for HN" if a neoplasm was favored by the pathologist or "FLUS" if there was a differential diagnoses of Hurthle cell neoplasm and hyperplastic lesion. Diagnoses of "Hurthle cell Nodule, favor hyperplasia" are excluded from the study.

Results: In comparison with the surgical resection specimens, 51 cases (50.4%) had good correlation between the cytology diagnosis and surgical excision, with 37 cases of Hurthle cell adenomas and 14 cases of Hurthle cell carcinomas identified. Fifty cases (49.6%) showed a discrepancy between the cytology diagnosis and resection. Twenty-four of the discrepant cases were papillary thyroid carcinoma (48%) on resection, including 9 follicular variants and 5 oncocytic variants. The second most frequent cause for discrepancy was Hurthle cell hyperplasia/adenomatoid nodules with oncocytic features, where 21 cases (42%) were identified. Three isolated discrepant cases of medullary carcinoma and 2 cases of poorly differentiated/insular carcinoma were also identified on surgical resection.

Conclusions: Hurthle cell lesions are still a challenging diagnosis for cytopathologists, where only 50% of the cases can be accurately diagnosed on cytology. The most common mistake made on cytology is due to papillary thyroid carcinoma (48%). The second most common cause for discrepant results is Hurthle cell hyperplasia (42%). Close clinical follow-up is necessary when Hurthle cell lesions are detected to avoid misdiagnosing an occult papillary thyroid carcinoma.

446 Liquid-Based Papanicolaou Tests in Endometrial Carcinoma Diagnosis

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Background: Currently, there is no cost effective screening tool for endometrial carcinoma. Conventional Pap smear is considered suboptimal for detection of endometrial carcinoma. However, morphology is superior in liquid-based Pap tests and their utility in detection of endometrial carcinoma has not been well studied. The aim of this study was to retrospectively examine the Pap test results of histologically diagnosed endometrial carcinoma patients prior to diagnosis.

Design: A retrospective search of our database was conducted (2006 to 2011) for women with histological endometrial carcinoma diagnoses. These cases were evaluated for the presence of previous Pap tests up to 36 months prior to diagnosis. The cases were evaluated for further clinicopathologic information (patient age, tumor type, FIGO grade, tumor size, myometrial invasion, angiolymphatic invasion, cervical invasion, FIGO stage, and previous Pap tests when present).

Results: A total of 222 cases of endometrial carcinoma were identified, including 186 endometrioid (83.8%), 14 mixed (5.9%), 8 serous (3.6%), 6 clear cell (2.7%), 2 mucinous (0.9%) carcinoma, and 5 MMMT (2.3%). Average patient age was 62 years (range= 33-91).126 patients (57.3%) had at least one Pap test up to 36 months prior to diagnosis. 84 of these 126 women had a Pap test in 4 months prior to histological diagnosis (Pap test results are summarized in Table 1): 28 (33.3%) had at least glandular abnormality, 8 (9.5%) had benign endometrial cells (all >40 years old), and 43 (51.2%) had negative results.

Pap test results prior to histopathological diagnoses of endometrial carcinoma (<5 months)

Categories	Case# (n=84)	%
Suspicious/malignant	13	15.5
AGC	13	15.5
AGC/SIL	2	2.4
Endometrial cells in >40 years old	8	9.5
ASC-US	5	6.0
Negative	43	51.2

The remaining 67 of 126 women had at least one Pap test in 5-36 months prior to carcinoma diagnosis: 53 (79.1%) had at least one negative Pap test, 8 (11.9%) had ASC-US, 5 (7.5%) had AGC, and 1 (1.5%) had ASC-H.

Conclusions: In our patient population of biopsy-proven endometrial carcinoma, 42.8% of cases had a Pap test with a diagnosis of at least AGC or endometrial cells over 40 years of age. This result demonstrates that Pap test plays a role in the detection of endometrial carcinoma. However, 51.2% of women had negative Pap tests up to 4 months before the malignancy diagnosis. Therefore, we conclude that Pap test is not a sensitive method for detection of endometrial carcinoma.

447 Validation of Cervista HPV16/18 Assay in Anal Swab Specimens

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Background: High-risk human papillomavirus (HPV) infection, specifically HPV16 and/or HPV18, is the major etiologic factor (>90%) for the development of anal carcinoma. In high-risk populations, such as HIV-positive patients, anal smears have been used for the surveillance of anal precancerous lesions, i.e. anal intraepithelial neoplasia (AIN). Similar to Pap Test in cervical specimens, HPV 16/18 testing may be useful for anal precancerous lesion surveillance. In this validation study, we evaluated Cervista HPV16/18, an FDA-approved HPV testing assay for Pap Test specimens, in anal swab specimens and compared results to PCR-based HPV16/18 testing results in split anal swab samples.

Design: Residual anal swab specimens (ThinPrep) were collected retrospectively from 98 patients (92 males and 6 females) at the Banner MD Anderson Cancer Center, Gilbert, AZ from 2011 to 2012. Patient's age ranged from 20 to 75 years old with a mean age of 43 years old. We performed HPV DNA testing using the Cervista HR and Cervista HPV 16/18 assays. Genotyping for HPV16 and HPV18 using a PCR-based HPV 16/18 genotyping assay was performed in the split samples of the anal swab. Cervista HPV 16/18 assay results were compared with PCR 16/18 genotyping results.

Results: Among the 98 specimens, 36 had insufficient genomic DNA for both Cervista HR and Cervista HPV 16/18 assays and were excluded from the data analysis. Of the 62 cases qualified for analysis, Cervista HR was positive in 42 (68%) cases. Cervista HPV16/18 was positive in 26 cases which included 12 cases of HPV18, 10 cases of HPV16 and 4 cases of HPV16 and HPV18. In 39 cases, there was sufficient DNA for both the Cervista HPV16/18 assay and the PCR-based HPV 16/18 genotyping assay. The results demonstrated a good correlation between the two HPV genotyping assays (kappa=0.69, 95% CI 0.46-0.92) (Table 1).

Table 1: Cervista HPV 16/18 and PCR-based HPV 16/18 genotyping in anal swab specimen (n=39)

PCR HPV 16/18	Positive	Negative	Total
Positive	17	4	21
Negative	2	16	18
Total	19	20	39

Kappa= 0.68

Conclusions: Cervista HPV 16/18 can be used as a valid HPV 16/18 genotyping assay in anal swab specimens.

448 Use of HBME-1, p27 and Galectin-3 Triple Immunostaining in Cytology and Histology Specimens To Help Clarify Follicular Lesions of Undetermined Significance (FLUS)

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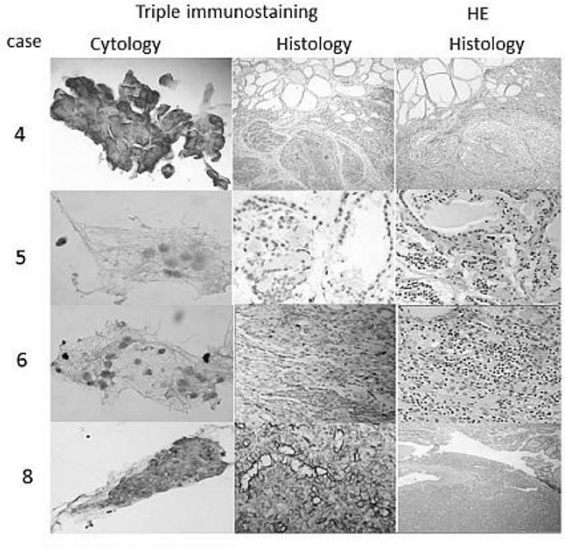
Background: Galectin-3 is the downstream target of PAX8/PPARγ translocation and HBME-1 is the downstream target of RAS activation. These two mutually exclusive pathways can lead to development of follicular carcinoma. Noteworthy, most papillary carcinomas express HBME-1, and some of papillary carcinomas grow as follicular-patterned lesions. The combined use of Galectin-3 and HBME-1 increases sensitivity of diagnosing follicular carcinoma and papillary carcinoma. P27 (kip1) is an inhibitor of G1/S cell cycle progression, and is highly expressed in normal thyroid follicular cells. Loss of p27 expression has been observed in over 85% of thyroid cancers. We hypothesize that Galectin-3, HBME-1 and p27 triple immunostaining would help clarify follicular lesions of undetermined significance of thyroid.

Design: A triple immunostaining protocol, using destained liquid based cytology (LBC) slides and formalin fixed paraffin embedded tissue (FFPE) slides, was developed in our lab. This protocol was applied to matched cytology and surgical pathology specimens retrospectively selected from 9 patients with a preoperative cytologic diagnosis of follicular lesion of undetermined significance (FLUS).

Results: The immunochemical staining pattern was similar in the matched LBC slides and the FFPE slides from the 9 patients. The 4 benign lesions (1 lymphocytic thyroiditis with adenomatoid hyperplasia, 2 adenomatoid nodules, 1 follicular adenoma) were negative (3/4) or scattered (<5%) positive (1/4) for HBME1/Galectin-3, and variably positive for p27 (4/4). The 5 malignant lesions (2 minimally invasive follicular carcinomas, 3 papillary carcinomas) were all diffusely positive for HBME1 and negative for p27 (5/5).



Conclusions: Our pilot study of triple immunostaining using a panel markers of HBME-1, Galectin-3 and p27 showed promising results in clarifying thyroid follicular lesions in both cytology and histology specimen. Further study is suggested to validate this assay.

449 Anal Cytologic Testing as a Screening Test – An Institutional Experience

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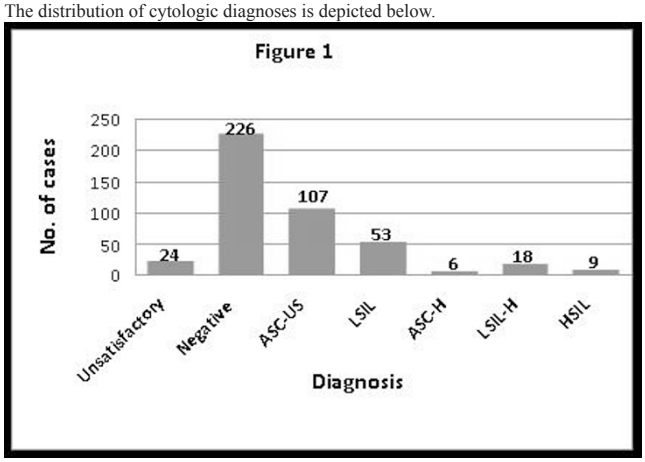
Background: Anal carcinoma incidence continues to rise, especially in men who have sex with men. The role of anal cytology in the early detection of high grade anal intraepithelial neoplasia (AIN) is still debatable and currently, there are no national

screening guidelines for anal cancer. Here, we describe our anal cytology experience, with regards to the patient population, the distribution of cytologic results, hrHPV (high risk human papillomavirus) detection rates and the histologic outcomes.
Design: A database search was conducted from January 2007 to June 2012 for anal cytologic tests (ThinPrep). Clinical information, the results of concurrent hrHPV testing (Hybrid Capture 2) if available, and histologic follow-up within the next 18 months, if any, were compiled. Also, the development of invasive squamous cell carcinoma during the study period was recorded. Statistical analysis was performed using chi-square test and Fisher's exact test and a p value of < 0.05 was considered as statistically significant.
Results: 443 patients had anal cytologic tests – average age 44.4 years (range 17-80 years), 74% HIV positive (318 men, 4 women), 26% HIV negative (56 men, 65 women). The distribution of cytologic diagnoses is depicted below.



Histologic follow-up was available in 173 (39%) patients. High-grade AIN or above was diagnosed in 30.2% ASC-US, 47.6% LSIL, 77.8% HSIL and 72.2% ASC-H/LSIL-H cases. The difference between the detection rates for high-grade AIN or above in the following groups was not statistically significant – ASC-US vs. LSIL, HSIL vs. ASC-H/LSIL-H and HIV positive vs. HIV negative patients. 76% ASC-US cases were hrHPV positive, of those that were tested. Invasive squamous cell carcinoma was diagnosed in 6 patients – 5 on immediate follow-up and one 33 months following an initial biopsy with AIN2, 3 were HIV positive, cytologic diagnoses included NILM (1), LSIL (2), LSIL-H (2) and HSIL (1).
Conclusions: Our study reveals a high rate of detection of high grade AIN following an abnormal cytology diagnosis and supports the management of patients with lesser cytologic abnormalities (ASC-US/LSIL) with high resolution anoscopy (HRA) and biopsy. Given the high hrHPV positivity rate in the ASC-US group, hrHPV testing may not play a significant role in the triage of these patients to HRA.

450 Vaginal Cytology and HPV Co-Testing Is Preferred for Follow-Up of Women with Invasive Cervical Cancer Treated by Hysterectomy

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Background: New cervical cancer screening guidelines indicate that women who have undergone hysterectomy and no history of cervical intraepithelial neoplasia (CIN) 2+ should not be screened for vaginal cancer. Women who have had a hysterectomy for invasive cervical cancer/CIN2+ may be at an increased risk of vaginal cancer, but data are very limited.
Design: A computer-based search of CoPath files was carried out to retrieve cases with invasive cervical carcinoma treated by hysterectomy with histopathologic and/or cytologic follow-up results during a study period of 10 years. Surgical pathology reports, follow-up hrHPV testing, cytologic, and histopathologic results were recorded.
Results: 147 patients with invasive cervical carcinoma [76 squamous cell carcinoma (SqCC), 60 adenocarcinoma (ADC) and 11 adenosquamous carcinoma] treated by hysterectomy and follow-up results were identified. The average age of these patients was 43 years (range: 29-72) at the time of diagnosis. The average follow-up period was 43.3 months (range: 3-139). Two cases (1.4%) of recurrent/residual SqCC were detected in vagina/vulva during follow-up, 1 and 11 months after the hysterectomy, respectively. 20 patients (13.6%) developed vaginal intraepithelial neoplasias (VAINs) during follow-up (table 1). The average interval between hysterectomy and initial diagnosis of VAIN2/3/HSIL (8 cases) was 8.6 months (range: 1-24). 47 women had hrHPV testing during follow-up and 29.7 % (11/47) had at least one positive hrHPV testing. Importantly, VIN was detected in 54.5% (6/11) of patients with hrHPV-positive result compared to 16.7% (6/36) of patients with negative hrHPV-negative result.

Vaginal cytologic and histologic follow-up results in 147 patients with invasive cervical carcinoma treated by hysterectomy.

	Case#	Carcinoma	VIN2/3/HSIL	VIN1/LSIL	Neg/ASC
Histology	34	2	7	9	16
Cytology only	113	0	1	3	109
Total	147	2	8	12	125

Legend: VIN: vaginal intraepithelial neoplasia; H/LSIL: high/low grade intraepithelial lesion; Neg: negative; ASC: atypical squamous cells.

Conclusions: VAIN1+ lesions were identified in 15.0% (22/147) patients with invasive cervical cancer treated by hysterectomy, indicating these women were at an increased risk of vaginal lesion. All VAIN2+ lesions were identified within 2 years after hysterectomy. The significantly increased detection rate of VAINs in hrHPV-positive group suggests vaginal cytology and HPV co-testing is preferred for follow-up of women with invasive cervical carcinoma after hysterectomy.

451 Non-16/18 HR-HPV Genotypes Are Superior to HPV 16/18 for Predicting High-Grade Intraepithelial Lesions in LEEP Resections

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Background: HPV 16 and 18 are responsible for the majority of cases of high-grade squamous intraepithelial lesions (HSIL) and cervical cancer. While HPV 16/18 have been extensively studied, more than a dozen non-16/18 high risk HPV (HR-HPV) genotypes have been overshadowed by the two leading HPV types and have received much less attention. With the current vaccines directed against HPV 16/18, non-16/18 HR-HPV genotypes are expected to play an increasing role in cervical cancer screening and prevention. Therefore, evaluating the predictive value of non-16/18 type HR-HPV is pertinent for early detection and cancer prevention, as well as for future vaccine development.
Design: A cohort of 808 SurePath specimens were collected from women who were referred to our institution from 12/2009-4/2011 for abnormal Pap tests. HPV genotypes were determined by DNA microarray against 40 HPV subtypes followed by a confirmatory sequencing assay. Forty-three patients from the cohort had a concurrent or subsequent LEEP procedure. Correlations among HPV genotype, cytologic findings and LEEP findings were analyzed.
Results: The HR-HPV infection rate was 81.4% in the 43 patients who underwent a LEEP and 94.4% in patients with HSIL on Pap tests. Cytologic interpretation of HSIL had 88.9% positive predictive value (PPV) for any grade of dysplasia and 83.3% for CIN 2 and above lesions in LEEPs. The PPV of non-16/18 HR-HPV for any grade of dysplasia in LEEPs was 93.3%, which was significantly better than that of HPV 16/18 (65%, $p=0.049$). For patients with CIN 2 and above lesions in LEEPs, the PPV of non-16/18 HR-HPV (73.3%) was also better than that of HPV 16/18 alone (58.3%). HPV 31/52/58/39/45 genotypes were commonly detected in women with HSIL in LEEP specimens. Furthermore, all patients with HPV31/52/58 infection documented on Pap test had HSIL in LEEP (100% positive predictive value).
Conclusions: The predictive value of non-16/18 HR-HPV genotypes for HSIL in LEEPs was superior to that of the HPV 16/18 test. The HPV16/18-only test may be not having as a high value as perceived, and a negative result may give a false assurance, especially in high risk populations. In addition to HPV 16/18, an expanded test panel of HR-HPV genotypes to include some of the non-16/18 genotypes with high PPV (such as HPV 31/52/58) may be warranted. At the present time, testing with a cocktail of HR-HPV types appears to offer a better predictive value for HSIL than using an HPV 16/18 test.

Dermatopathology

452 The Impact on Final Breslow Thickness and Sentinel Lymph Node Status with Initial Biopsies of Cutaneous Melanomas Transected at the Base

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Background: The histologic evaluation of primary tumor characteristics (Breslow thickness (BT), mitotic rate (MR), ulceration) in cutaneous melanoma is critical for staging and classification, prognosis and clinical management decisions. BT is a strong and consistent histologic parameter of sentinel lymph node (SLN) status. Melanomas that are superficially sampled with tumor cells present at the deep tissue edge may underestimate true BT, pose a risk for positive SLNB, and provide inaccurate prognostic information. We report the impact of positive deep margins on initial biopsies of cutaneous melanoma with respect to final BT on wide local excision (WLE) and outcome of SLNB.
Design: An eighteen month retrospective review was performed in patients diagnosed with primary cutaneous melanoma who undergone WLE and SLNB. Patients were categorized into four groups according to status of deep margin on initial biopsy and outcome of SLNB. G1=absence of positive deep margin and negative SLNB, G2=absence of positive deep margin and positive SLNB, G3=positive deep margin and negative SLNB, G4=positive deep margin and positive SLNB. Comparisons between groups were made with Kruskal Wallis test. Statistical significance was considered at $p < 0.05$.
Results: 171 patients fulfilled the criteria and were distributed into the four categories as follows: G1 (70), G2 (31) G3 (51), G4 (19). Groups with positive SLNB (G2 & G4) had greater final BT than patients with negative SLNB (G1 & G3) regardless of the status of deep tissue edge on initial skin biopsy. The final BT in patients in G4 patients was significantly greater compared to the final BT in the other groups. G4 patients also had greater tumor deposit size in SLNB compared to G2 patients.

Table 1

	G1	G2	G3	g
CL median (range)	4 (2-5)	4 (4-5)	4 (2-5)	4 (3-5)
Initial BT mm* (range)	2.4(0.4-17)	3.12(0.54-15)	2.19(0.20-9.7)	2.86(0.57-5.0)
Final BT mm* (range)	2.55(0.44-17)	3.18(0.54-15)	2.60(0.20-9.7)	3.38(1.5-5.0)
Tumor deposit size (mm)* in SLN* (range)	NA	1.63(0.1-5.3)	NA	2.77(0.1-15)

* Average values, $p < 0.05$

Conclusions: Initial biopsies of melanoma with positive deep margins demonstrate risk for greater final BT and increase tumor burden in SLNB. Appropriate initial sampling of melanoma is important in staging since superficially sampled tumors may limit accurate evaluation of histologic parameters which may have significant prognostic implications.