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ABSTRACTS

CYTOPATHOLOGY

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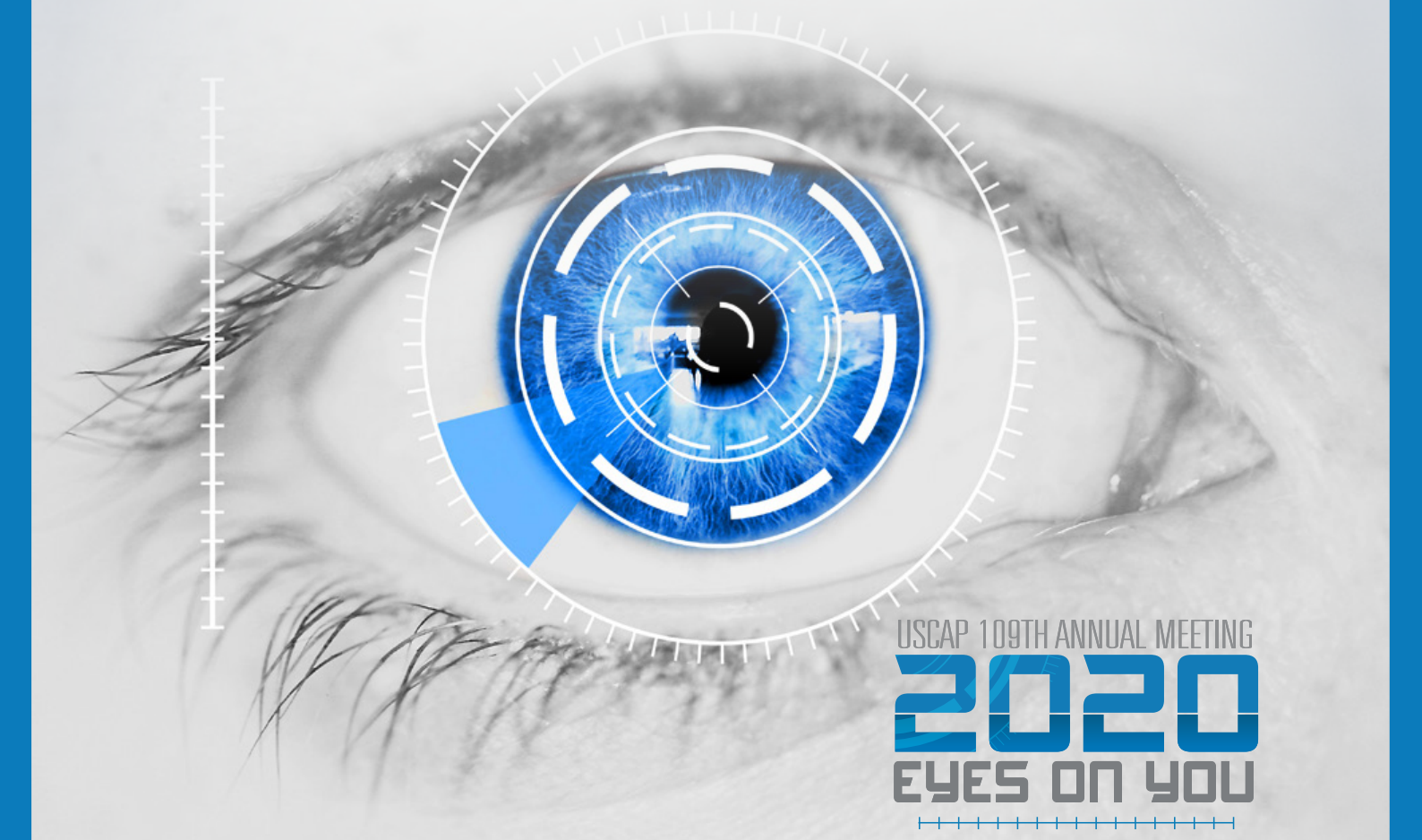

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317 Tumor Diagnosis Using Intraoperative Imprint Cytology (IIC) in a Peruvian Cancer Center. A Seven-Year Experience

Milagros Abad-Licham[1], Juan Astigueta[1], Ricardo Bardales[2], Jesus Gonzales-Saravia[1], Cynthia Ortiz[1], Oriana Vasquez[1], Luis De Las Casas[3]
[1]North Regional Cancer Center, Trujillo, La Libertad, Peru, [2]Outpatient Pathology Associates, Sacramento, CA, [3]University of Rochester Medical Center, Rochester, NY

Disclosures: Milagros Abad-Licham: None; Juan Astigueta: None; Ricardo Bardales: None; Jesus Gonzales-Saravia: None; Cynthia Ortiz: None; Oriana Vasquez: None; Luis De Las Casas: None

Background: Intraoperative cytologic interpretation has emerged as an accurate, simple, and rapid tool for tumor diagnosis. Well-prepared cytologic samples are as accurate as frozen sections during the intraoperative evaluation of tumors. The diagnostic accuracy of this technique depends on the pathologist experience and dedication to prepare and interpret the cytologic sample.

Design: A seven-year (2012-2018) retrospective study of cases with IIC with tissue correlation at the North Regional Cancer Center of Trujillo, Peru was performed. Patient's demographic data, IIC, and surgical pathology reports were collected, analyzed, and tabulated. Imprint cytology (without concurrent frozen section) was exclusively used to make intraoperative diagnoses of 1814 surgically removed tumors from 887 patients [80% (704/887) women, 20% (183/887) men; $\bar{x}$: 53 years; range: 9-93 years)]. Tumor sites included: lymph nodes (n=887, 49%), ovary and endometrium (n=178, 9.8%), thyroid (n=110, 6%), peritoneum (n=115, 6.3%), liver and pancreatobiliary (n=46, 2.5%), gastrointestinal (n=26, 1.4%), retroperitoneum (n=19, 1%), penis (n=12, 0.6%), and miscellaneous (n=421, 23%). Three experienced pathologists prepared and diagnosed the IIC in all cases.

Results: IIC diagnoses include 1234 benign (68%), 478 malignant (26%), 68 atypical (4%), and 34 non-diagnostic/deferred cases (2%). Final tissue diagnoses included 1288 benign/non-malignant (70%) and 526 malignant (30%) tumors/masses. IIC rendered definitive interpretations in 1712 of the 1814 cases (94%). There was concordance with histologic diagnosis in 1638 of 1712 cases (96%), while the remaining 74 (4%) cases represent discrepancies attributed to sampling or interpretative errors. The most significant discrepancy was that of an endometrial biopsy in a 80-year-old female diagnosed as endometrial adenocarcinoma on IIC and histologically diagnosed as endometrial adenofibroma. The use of IIC for tumor diagnosis has an overall sensitivity of 94%, specificity 97%, positive predictive value 92%, and negative predictive value 98%.

Conclusions: Our results support the use of imprint cytology as a valuable alternative for intraoperative diagnostic interpretations when frozen section is not available.

318 How Lymphoma Diagnosis by Fine Needle Aspiration Cytology Works in a Tertiary Care Medical Center

Marcia Abbott[1], Liuyan (Jennifer) Jiang[2], Bahaaeldin Youssef[3], Ahsan Siddiqi[4], Aziza Nassar[4]
[1]Calgary Laboratory Services/University of Calgary, Calgary, AB, [2]Mayo Clinic Florida, Jacksonville, FL, [3]East Tennessee State University, Johnson City, TN, [4]Mayo Clinic, Jacksonville, FL

Disclosures: Marcia Abbott: None; Liuyan (Jennifer) Jiang: None; Bahaaeldin Youssef: None; Ahsan Siddiqi: None; Aziza Nassar: None

Background: The diagnosis of lymphoma often presents a challenge because of the high number of diagnostic categories and the increasing reliance of hematopathology on tissue-intensive supplemental analyses. Herein, we describe our institutional experience with patients who ultimately went on to carry a diagnosis of lymphoma and detail the completeness and confidence in the cytologic diagnosis that could be rendered from the fine needle aspiration (FNA) material alone.

Design: The electronic laboratory information system was queried for FNA that resulted in the diagnosis of lymphoma, atypical lymphoid population, or suspicious for lymphoma. In formal and suspicious for lymphoma diagnoses, the classifications followed the established nomenclatures of the World Health Organization. For all cases, the ancillary test profiles were captured and summarized.

Results: 389 cases were identified with a mean age of 66.0 years (range: 21.0 – 98.0). Seventeen cases (17/389 – 4.4%) were given the diagnosis of atypical lymphoid cells; whereas 31 cases (31/389 – 7.9%) were suspicious for lymphoma. This has an overall indeterminate rate of 12.3%. The rest of the malignant cases (341/87.7%) were classified using the 2016 WHO lymphoid classification system (see attached Table). There are six low-grade and 26 high-grade B-cell lymphomas that were not classified further due to limited tissue. B-cell lymphomas are the largest category (284/389 – 73.0%), with the leading diagnosis of DLBCL (84 – 21.4%), followed by FL (68 – 17.5%) and CLL/SLL (43 – 10.9%). Three hundred nineteen (82.0%) of the cases had baseline IHC staining panel performed using a combination of 28 hematologic specific stains diagnoses. These cases had robust flow cytometry results (140/389 – 36.0%), but 30 (21.4%) specimens were insufficient. There were a low number of cases which showed typical FC immunophenotypic results for acute leukemia (1/140 = 0.7%), T-cell leukemia (1/140= 0.7%), and polytypic B-cells (4/140 = 2.9%). This left the majority of cases that were sufficient with typical B-cell immunophenotypic profile (104/140 = 74.3%).

Seventy-eight (78/389 – 20.0%) cases had cytogenetic and molecular studies, FISH (for c-myc, Bcl-2 and Bcl-6), immunoglobulin gene and T-cell receptor rearrangement studies.

Table 1. Classification

	A	B	C
1	**DIAGNOSES AS PER 2016 WHO CLASSIFICATION**	**# CASES**	**% CASES**
2	**BLASTIC PLASMACYTOID DENDRITIC CELL NEOPLASM**		
3	Blastic plasmacytoid dendritic cell neoplasm	1	0.3
4	**PRECURSOR LYMPHOID NEOPLASMS**		
5	B-cell lymphoblastic leukemia/lymphoma	5	1.3
6	T-cell lymphoblastic leukaemia/lymphoma	4	1
7	**MATURE B-CELL NEOPLASMS**		
8	***LOW GRADE***		
9	Chronic lymphocytic leukaemia/small lymphocytic lymphoma	43	11.1
10	Lymphoplasmcytic lymphoma	9	2.3
11	Plasmacytoma	7	1.8
12	Extranodal marginal zone lymphoma	1	0.3
13	Marginal zone lymphoma	17	4.4
14	Follicular lymphoma, any grade	68	17.5
15	Mantle cell lymphoma, including blastoid variant	16	4.1
16	Low grade B-cell lymphoma, NOS	6	1.5
17	***HIGH GRADE***		
18	Diffuse large B-cell lymphoma, all variants	84	21.6
19	Primary mediastinal large B-cell lymphoma	1	0.3
20	Plasmablastic lymphoma	3	0.8
21	Primary effusion lymphoma	1	0.3
22	Burkitt's lymphoma	2	0.5
23	High grade B-cell lymphoma, NOS	26	6.7
24	**MATURE T-CELL AND NK-CELL NEOPLASMS**		
25	T-cell prolymphocytic leukemia	3	0.8
26	Mycosis fungoides	1	0.3
27	Sezary syndrome	1	0.3
28	Peripheral T-cell lymphoma, NOS	3	0.8
29	Angioimmunoblastic T-cell lymphoma	3	0.8
30	Anaplastic large-cell lymphoma	3	0.8
31	**HODGKIN LYMPHOMA**		
32	Hodgkin lymphoma, NOS	8	2.1
33	Classical Hodgkin lymphoma	17	4.4
34	Nodular sclerosis classical Hodgkin lymphoma	5	1.3
35	Lymphocyte-rich classical Hodgkin lymphoma	1	0.3
36	Mixed cellularity classical Hodgkin lymphoma	1	0.3
37	**POST-TRANSPLANT LYMPHOPROLIFERATIVE DISORDERS**		
38	Post-transplant lymphoproliferative disorder	1	0.3
39	**SUSCPICIOUS FOR VARIOUS TYPES LYMPOMA**		
40	Suspicious for lymphoma	31	8
41	**VARIOUS ATYPICAL LYMPHOID CELLS**		
42	Atypical cells	17	4.4
43		389	100.7

Figure 1 - 318

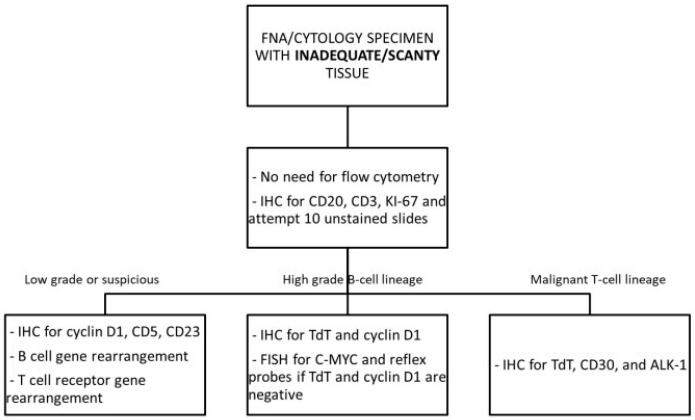
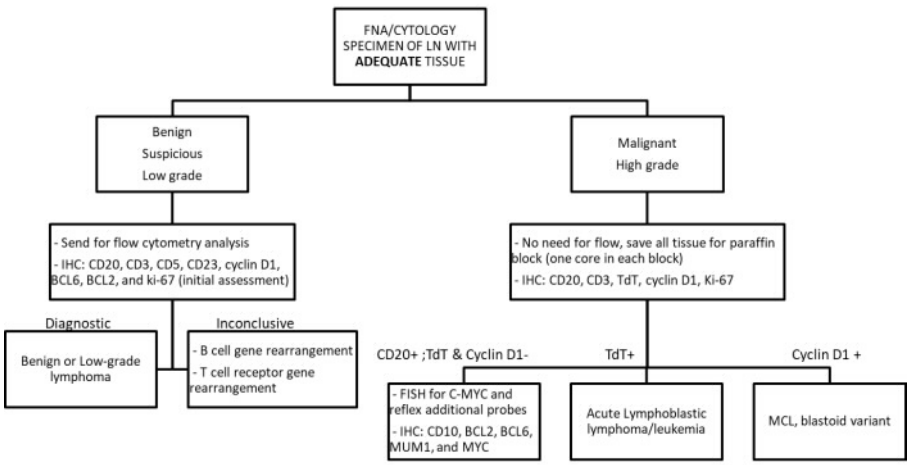


Figure 2 - 318



Conclusions: With some predictable exceptions, full classification of lymphomas is possible with FNA material, and given its ease and convenience, it is an attractive first sampling modality in the diagnostic process for this disease group.

319 Histologic Follow-up Results in Women with Atypical Glandular Cells in Pap Test: A Five-Year Single Institution Experience

Elizabeth Abels[1], Ranjana Nawgiri[2], Jing He[3], Cecilia Clement[2]
[1]University of Texas Medical Branch - UTMB, Galveston, TX, [2]University of Texas Medical Branch, Galveston, TX, [3]University of Texas Medical Branch, Friendswood, TX

Disclosures: Elizabeth Abels: None; Ranjana Nawgiri: None; Jing He: None; Cecilia Clement: None

Background: Atypical glandular cells (AGCs) in the Papanicolaou (Pap) test may be associated with significant gynecologic squamous and glandular abnormalities. Several studies have addressed the incidence and clinical implications of AGC diagnosis with varied results. The aim of this study is to evaluate the final histological diagnoses of women with Pap test interpreted as AGC, and to identify clinical characteristics of this study group.

Design: We retrospectively reviewed all Pap test cases interpreted as AGC performed in our institution between 2014 and 2018. Cases with histologic follow-up diagnoses were identified. Clinical characteristics including age, race/ethnicity, symptoms, menopausal status and high-risk human papillomavirus (HR-HPV) result were evaluated.

Results: Cervical Pap was performed in 95,000 patients. A diagnosis of AGC was made on cytology in 135 (0.14%) patients, consistent with the reported range in the literature (0.08-2.1%), and 105 of those received histological work-up and were included in our study. There were significant pathologic lesions in 40 (38.1%) women and these include 22 (20.4%) endometrial, 6 (5.6%) cervical-squamous, 9 (8.3%) cervical-glandular, 1 (0.9%) cervical-adenosquamous and 2 (1.9%) other genital lesions. The median age and range of the study population was 50 years and 24-80, respectively. Among those with significant lesions the median age and range was 51 years and 26-75 years, respectively. Age, ethnicity and menopausal status were not associated with higher rates of significant lesions while presence of symptoms (defined as any abnormal uterine bleeding) and AGC-favor neoplasia (AGC-FN) category were more likely to be associated with significant lesions. See **Table 1** for detailed subgroup analysis.

Significant histology according to patient characteristics and cytology subgroups

		Significant histology identified n (%)	No significant histology identified n (%)
Cytology			
	AGC-NOS	9 (28.1%)	23 (71.9%)
	AEM-NOS	10 (21.3%)	37 (78.7%)
	AEC-NOS	6 (54.6%)	5 (45.5%)
	AEC-FN	4 (80.0%)	1 (20%)
	AGC-FN	11 (84.6%)	2 (15.4%)
Age			
	< 30	2 (40%)	3 (60%)
	30-50	17 (36.2%)	30 (63.8%)
	≥50	21 (37.5%)	35 (62.5%)
Ethnicity			
	Asian	2 (66.7%)	1 (33.3%)
	Black	7 (36.8%)	12 (63.2%)
	Hispanic	10 (32.3%)	21 (67.7%)
	White	21 (39.6)	32 (60.4%)
Menopausal Status			
	Pre	20 (33.9%)	39 (66.1%)
	Post	20 (41.7%)	28 (58.3%)
Symptomatic			
	Yes	28 (44.4%)	35 (55.6%)
	No	12 (26.7%)	33 (73.3%)
HPV Status			
	Positive	9 (60%)	6 (40%)
	Negative	18 (28.1%)	46 (71.9%)
	Unknown	13 (44.8%)	16 (55.2%)

AGC: Atypical glandular cells, AEM: Atypical glandular cells of endometrial origin, AEC: Atypical glandular cells of endocervical origin, NOS: Not otherwise specified, FN: Favor neoplasia

Conclusions: Approximately one third of all women with AGC on Pap were found to have dysplastic lesions or malignancies. However, age, ethnicity and menopausal status were not predictive of a significant histologic result. Presence of symptoms and a finding of AGC-FN were the only factors predictive of significant histologic results. This suggests that identification of AGCs in a Pap test is clinically important in all patients, especially those who present with abnormal uterine bleeding and Pap with AGC-FN. Histologic workup should be pursued in all instances of Pap positive for AGC.

320 Fine Needle Aspiration Cytomorphology of Papillary Thyroid Carcinomas with NTRK Gene Rearrangement

Rita Abi-Raad[1], Manju Prasad[1], Adebowale Adeniran[2], Guoping Cai[3]
[1]Yale University School of Medicine, New Haven, CT, [2]Yale University School of Medicine, Seymour, CT, [3]Yale University, Wallingford, CT

Disclosures: Rita Abi-Raad: None; Manju Prasad: None; Adebowale Adeniran: None; Guoping Cai: None

Background: Neurotrophic tyrosine kinase receptor (NTRK) gene rearrangement has been reported in a subset of papillary thyroid carcinoma (PTC), mostly occurring in children or patients with radiation exposure. Recognition of these PTCs in fine needle aspiration (FNA) may be important to appropriately manage those patients. We aim to study the cytomorphologic features of NTRK-rearranged PTCs in FNA specimens from non-irradiated patients.

Design: 13 FNA specimens of PTC with NTRK rearrangement were retrieved from the pathology archives between January 1, 2010 and June 30, 2019. NTRK gene rearrangement was detected on FNA samples or follow-up surgical resection specimens by next generation sequencing technique. Patients' demographic information, FNA cytologic characteristics and surgical follow-up were retrospectively reviewed.

Results: There were 10 female and 3 male patients with a median age of 18 years. The original cytological diagnoses included follicular lesion of undetermined significance (n=5; 38%), follicular neoplasm (n=1; 8%), suspicious for PTC (n=4; 31%) and PTC (n=3; 23%). Surgical follow-up was available in 12 cases, with classic PTC in 3 cases, follicular variant (FVPTC) in 4 cases, solid variant in 3 cases, diffuse sclerosing variant and mixed classic PTC and FVPTC in one case each. NTRK3 gene rearrangement was identified in 12 cases, with the partner gene as ETV6 in 11 cases and unknown in 1 case. NTRK1/TPR gene fusion was identified in the last case. Of 11 FNA specimens available for review, the cellularity was moderate or high in 10 cases and thick colloid was present in 8 cases. The architectures included papillary groups, loosely cohesive groups, mixed micro and macrofollicles, and single plasmacytoid cells in 4, 9, 9 and 11 cases, respectively. The cytoplasm was often granular (10 cases) and nuclear features were often subtle. The cytomorphologic characteristics are summarized in Table 1. Interestingly, lymphocytic thyroiditis was noted in the background of all cases.

Table 1. Cytomorphologic features of PTC with NTRK gene rearrangement

		n (%)
Cellularity	High	4 (36)
	Moderate	6 (55)
	Low	1 (9)
Architecture	Papillary groups	4 (36)
	Loosely cohesive groups	9 (82)
	Mixed micro/macro follicles	9 (82)
	Single cells	11 (100)
Cytoplasm	Vacuolated	3 (27)
	Dense	2 (18)
	Granular	10 (91)
Chromatin	Coarse	7 (64)
	Fine	4 (36)
Prominent nucleoli	Yes	6 (55)
	No	5 (45)
Nuclear elongation	Yes	11 (100)
	No	0 (0)
Nuclear membrane irregularity	Yes	9 (82)
	No	2 (18)
Nuclear grooves	Yes	10 (91)
	No	1 (9)
Intranuclear inclusions	Yes	1 (9)
	No	10 (91)
Nuclear enlargement	Yes	11 (100)
	No	0 (0)

Conclusions: In our series, the majority of PTC with NTRK rearrangement were non-classic PTC subtypes. The cytomorphologic features appear unique, characterized by the presence of loosely cohesive groups and single plasmacytoid cells with fine to granular cytoplasm and subtle nuclear features. Recognition of these cytomorphologic features in FNA specimens may help prompt molecular testing, leading to a preoperative diagnosis of this specific entity.

321 High-Risk HPV (hrHPV) Testing, Genotyping and Histopathologic Follow-up in Women with Atypical Squamous Cells - Cannot Exclude High-Grade Squamous Intraepithelial Lesion (ASC-H) on Pap Testing

Rita Abi-Raad[1], Deborah Barlow[2], Kara Duch[2], Angelique Levi[3], Malini Harigopal[1], Guoping Cai[4]
[1]Yale University School of Medicine, New Haven, CT, [2]Yale University, New Haven, CT, [3]Yale University, Fairfield, CT, [4]Yale University, Wallingford, CT

Disclosures: Rita Abi-Raad: None; Deborah Barlow: None; Kara Duch: None; Angelique Levi: None; Malini Harigopal: None; Guoping Cai: None

Background: According to the American Society for Colposcopy and Cervical Pathology (ASCCP) guidelines, women with ASC-H should undergo colposcopy regardless of hrHPV result. Reflex hrHPV testing is not recommended due to the high positive hrHPV rate in ASC-H patients and relatively high 5-year cancer risk among those even with negative hrHPV results. However, inter-observer concordance of ASC-H is relatively low and thus the risk of Cervical Intraepithelial Neoplasia 2 and above (CIN2+) on biopsy varies significantly. Herein, we review whether reflex hrHPV testing/genotyping helps triaging a subset of patients with ASC-H who may be spared colposcopy.

Design: We retrospectively reviewed cases with a PAP diagnosis of ASC-H with hrHPV cotesting and genotyping performed between 2016 and 2018. Only patients with pertinent histologic follow-up were included in the study.

Results: The study included 498 patients with ages ranging from 18 to 75 years old (394 patients ≤50 years old and 104 patients > 50 years old). A total of 189 patients had at least CIN2 on biopsy (38%) with a median follow-up of 37 days (0-742 days). Of patients ≤50 years old, 72 (18%) had negative hrHPV while 322 (82%) had positive hrHPV; 15 (21%) negative hrHPV and 149 (46%) positive hrHPV patients were found to have CIN2+ on follow-up biopsy (p=0.0002). Of patients > 50 years old, 32 (31%) had negative hrHPV, and 72 (69%) had positive hrHPV; 2 (6%) negative hrHPV and 23 (32%) positive hrHPV patients had CIN2+ on follow-up biopsy (p=0.0054). Of positive hrHPV cases, 114 were HPV 16/18, and 280 were non-16/18 HPV. In patients ≤ 50 years old, the risk of CIN2+ was not significantly different between HPV 16/18 and HPV non- 16/18 groups (54% and 43%; p=0.1). In patients > 50 years old, the risk of CIN2+ was the same at 32% in both groups.

Table 1. Impact of hrHPV testing and genotyping on detecting CIN2+ in patients with ASC-H on pap test

Age Group (years)	hrHPV	CIN2+ n (%)	HPV genotype	CIN2+ n (%)
≤ 50	Negative (n=72)	15 (21)	n/a	
	Positive (n=322)	149 (46)	HPV 16/18 (n=89)	48 (54)
			HPV non 16/18 (n=233)	101 (43)
> 50	Negative (n=32)	2 (6)	n/a	
	Positive (n=72)	23 (32)	HPV 16/18 (n=25)	8 (32)
			HPV non 16/18 (n=47)	15 (32)

Conclusions: Our results demonstrate that patients >50 years old with ASC-H but negative hrHPV have a very low risk of CIN2+ and may be safely managed with clinical follow-up and repeat PAP. HPV genotyping does not offer additional benefits for risk stratification in patients with ASC-H and positive hrHPV.

322 Does Reprocessing of Gynecological Cytology Sustain Increased Risk of Overinterpretation?

Schuharazad Abro[1], Stefan Pambuccian[1], Güliz Barkan[2], Eva Wojcik[1], Mohammed Atieh[1], Swati Mehrotra[1], Grazina Chatt[1]
[1]Loyola University Medical Center, Maywood, IL, [2]Loyola University Healthcare System, Maywood, IL

Disclosures: Schuharazad Abro: None; Stefan Pambuccian: None; Eva Wojcik: None; Mohammed Atieh: None; Swati Mehrotra: None

Background: An unsatisfactory Pap Test (PT) is unreliable for detection of cervical epithelial abnormalities. Insufficient cellularity, obscuring inflammation and blood account for the common reasons for unsatisfactory specimens. Several laboratories have resorted to an acetic-acid wash to the PT and found significant improvement in the unsatisfactory rate and increased detection of cervicovaginal abnormalities. The present study was undertaken to determine the detection rate of significant cervicovaginal abnormalities in the cohort of reprocessed specimen's vs all gynecologic cytology specimens processed during a period of one year.

Design: The institutional cytology database was queried for the period of 1year (2017) to extract data for all reprocessed gynecologic cytology samples. All samples were processed using the ThinPrep® PT. The reprocessing was performed using 2% glacial acetic acid. Histologic follow up, cytohistologic correlation and HPV results of reprocessed samples were compared with those of total gynecological cohort. Fisher exact test or Chi square with the each correction was used to determine statistical significance.

Results: There were a total of 10858 samples of which 730 (6.7%) were reprocessed. Histologic follow up was available in 66 (9.0%) for reprocessed samples and 384 (3.6%) of total ThinPrep samples. The diagnostic rates (ASCUS and above) were comparable (83/730, 11.4% vs 112/10858, 10.3 %, p= 0.4). The HPV positive in cases of ASCUS and above were slightly higher in all cases than in a reprocessed ThinPrep (15/67, 23.39% vs 191/524, 36.45%, p 0.02). However this may be due to the older mean age women with reprocessed specimen. The histological follow up diagnoses of CIN1+ (excluding adenocarcinoma) for the cases diagnosed as ASCUS and above did not defer significantly between the two cohorts (73/451, 16.2% vs 4/29, 13.8%, p value 0.06). There was a significant higher rate of adenocarcinomas in the reprocessed LBP (4/29, 13.8% vs 5/451, 1.1%, p= 0.001). See Table 1

Figure 1 - 322

Table 1

Reprocessed ThinPrep	DIAGNOSTIC RATE		HPV		FOLLOW UP (n=66, 9.0%)						
					LSIL		HSIL+		Other		Total
n= 730 (6.7%)	Number	%	Number	%	Number	%	Number	%	Number	%	
UNSAT	84	11.50%	1	2.27%	1	20.00%	0	0.00%	1	20.00%	5
Negative	563	77.10%	23	6.22%	1	3.13%	0	0.00%	3	9.38%	32
ASCUS	53	7.30%	9	18.37%	2	20.00%	1	10.00%	0	0.00%	10
LSIL	6	0.80%	3	100.00%	1	100.00%	0	0.00%	0	0.00%	1
HSIL+	6	0.80%	1	33.33%	1	20.00%	2	40.00%	0	0.00%	5
Other	18	2.50%	2	16.67%	0	0.00%	2	15.38%	5	38.46%	13
Total ThinPrep	DIAGNOSTIC RATE		HPV		FOLLOW UP (384, 3.6%)						
					LSIL		HSIL		Other		Total
n= 10858	Number	%	Number	%	Number	%	Number	%	Number	%	
UNSAT	107	1.00%	38	2.56%	0	0.00%	0	0.00%	2	40.00%	5
NEG	9628	91.40%	4857	5.32%	24	6.74%	9	2.53%	2	0.56%	356
ASCUS	647	6.10%	258	27.73%	26	32.91%	14	17.72%	0	0.00%	79
LSIL	298	2.80%	41	56.38%	35	18.32%	15	7.85%	0	0.00%	191
HSIL+	116	1.10%	16	44.83%	9	5.81%	33	21.29%	0	0.00%	155
OTHER	62	0.60%	18	40.91%	3	11.54%	2	7.69%	4	15.38%	26

Conclusions: We found no significant difference in the rate of diagnostic abnormalities (ASCUS and above) and in follow up histologic diagnosis other than adenocarcinomas between reprocessed PT and all PT. This may be attributed to experience of the cytopathologists who are aware of pitfall of cytologic changes induced by reprocessing.

323 Sarcoidosis: An Underappreciated Cause of Lymph Node Abnormality in Patients with Malignancy

Ashna Aggarwal[1], Friederike Kreisel[2], Hannah Krigman[3]
[1]Barnes Jewish Hospital/Washington University, St. Louis, MO, [2]Washington University, St. Louis, MO, [3]Washington University School of Medicine, St. Louis, MO

Disclosures: Ashna Aggarwal: None; Friederike Kreisel: None

Background: An association between sarcoidosis and malignancy was first reported in 1972 in a series of sarcoidosis (SRC) and sarcoid-like reactions (SLR) in patients with Hodgkin lymphoma and other non-Hodgkin lymphomas. Sarcoidosis is also linked to solid cancers; the latter has mostly been published as single case reports. In our experience, a significant number of patients evaluated for lymphadenopathy or PET avid lymph nodes in the setting of prior malignancy have non-necrotizing sarcoid-like granulomas on sampling by cytology. We sought to investigate the frequency and type of malignancies associated with nodal sarcoidosis.

Design: A retrospective review of our pathology database from 2001 – 2018 identified 127 patients with non-necrotizing granulomas on cytology. The clinical charts for all these patients were reviewed for patient characteristics, presence and type of malignancy, established diagnosis of SRC and the time interval between malignancy and development of granulomas.

Results: Of the 127 patients with non- necrotizing granulomas, 67 cases (52.75%) were associated with malignancy. 46 (68.65%) of these were discovered during staging of the malignancy, 14 (20.89%) were discovered after treatment during patient surveillance. 7 patients (10.44%) had a diagnosis of SRC; 2 patients carried a diagnosis of SRC prior to diagnosis of malignancy and 5 patients were diagnosed with SRC in the course of staging or followup. The most common malignancies associated with SLR were lung cancer (35.82%), gastrointestinal cancer (22.38%) and non-Hodgkin lymphomas (10.4%).

Conclusions: Sampling of lesions suspicious for metastatic malignancy during staging and surveillance has led to a heightened awareness of SRC and SLR associated with malignancy. It remains unclear whether this change represents an incidental finding or may be a form of immune response to the malignancy.

324 Body Fluid Supernatants Contain Sufficient Cell-Free DNA for Targeted Next Generation Sequencing

Naomi Akagi[1], Roberto Ruiz-Cordero[2], Dianna Ng[3], Joshua Menke[4]
[1]University of California San Francisco, San Francisco, CA, [2]University of California San Francisco Pathology, San Francisco, CA, [3]University of California San Francisco Medical Center, San Francisco, CA, [4]Millbrae, CA

Disclosures: Naomi Akagi: None; Roberto Ruiz-Cordero: None; Dianna Ng: *Primary Investigator*, Cepheid, Inc.; Joshua Menke: None

Background: In this era of targeted therapeutics and minimally-invasive testing, liquid-based samples are emerging as an important adjunct, or even alternative, to solid tumor biopsies for molecular tests. Liquid-based specimens, such as body fluids, are less invasive to collect. While supernatants are often discarded, they can contain adequate DNA for evaluation with next generation sequencing (NGS) to provide clinically actionable mutation profiles. Our primary aim is to evaluate a plasma circulating cell-free DNA (cfDNA) extraction method for obtaining sufficient tumor DNA from body fluid supernatants to perform a targeted 500 gene NGS panel. A secondary aim is to quantify DNA yields from benign effusions with the goal of detecting low-level, occult neoplasms or infections.

Design: DNA from 1 ml of 25 body fluid supernatant samples (8 malignant, 17 benign) was extracted using the Maxwell RSC ccfDNA Plasma Kit (Promega Corporation). Cell pellet samples from 6 of the 8 malignant samples were extracted for comparison to the supernatants. DNA yield (ng) was quantified using a Qubit fluorometer (ThermoFisher Scientific). A minimum of >50 ng of DNA was required for the NGS panel. Cell pellets were generated from 15 ml of the sample. Tumor cellularity was estimated visually on the direct stained smears.

Results: Malignant supernatants yielded a median 679 ng of DNA (66 to >3000 ng) from only 1 mL samples. Corresponding cell pellets showed a median of 1180 ng of DNA (118 to >3000 ng), 501 ng higher on average compared to supernatant. Estimated median tumor percentage was 70% (10 to 100%). Malignant supernatant volumes ranged from 7 to 2000 mL.

Benign supernatants yielded a median of 109 ng of DNA (2.6 to >3000 ng). The supernatant yielded sufficient DNA (50 ng) for NGS in 19/25 (76%) cases (8 malignant, 11 benign). All six cell pellets contained sufficient DNA for NGS. Three benign and one tumor sample yielded DNA at levels too high for Qubit quantification; on review, benign cases showed abundant inflammation, but the tumor case showed virtually none.

Figure 1 - 324

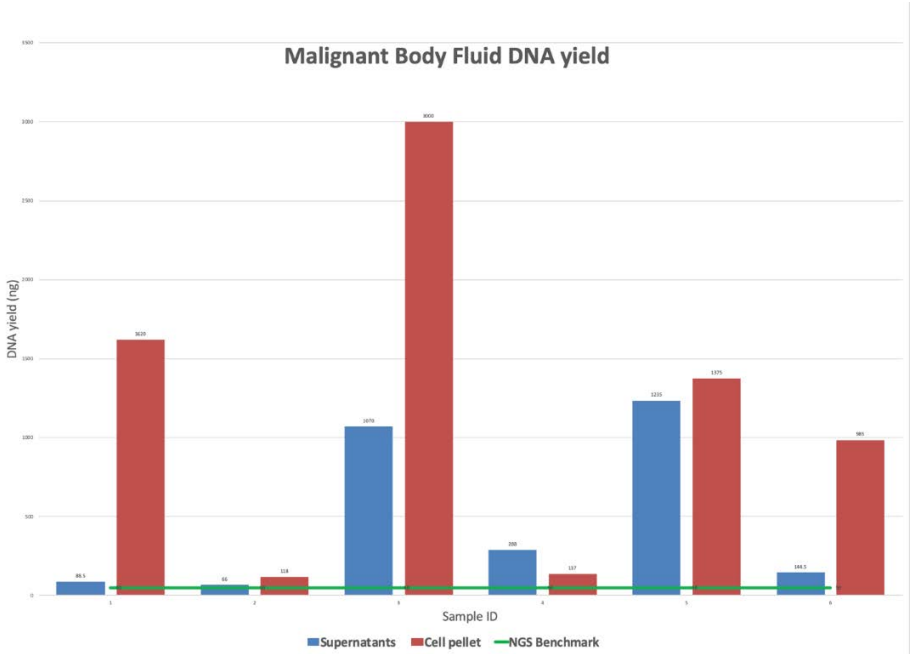


Figure 2 - 324

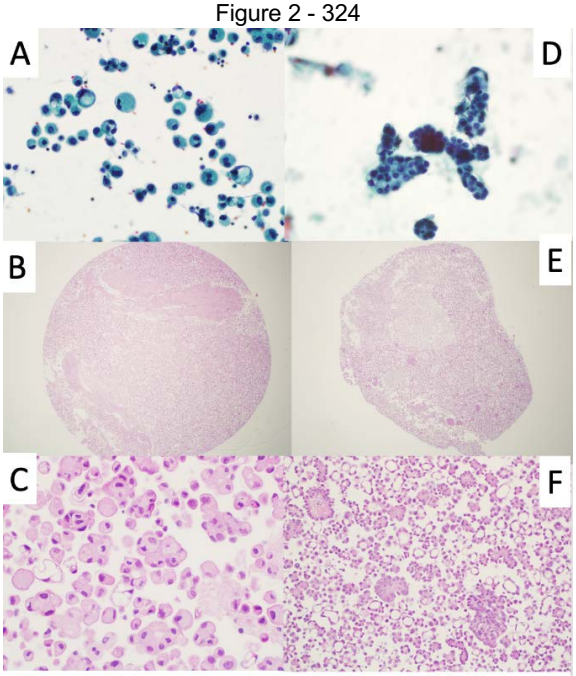


Figure 1. On left, breast carcinoma in pleural fluid A. Pap stain. B. Cell block H&E 20X. C. Cell block H&E 400X. On right, pulmonary adenocarcinoma in pleural fluid. D. Pap stain. E. Cell block H&E 20X. F. Cell block H&E 200X.

Conclusions: All malignant supernatants showed adequate cfDNA for a complete 500 gene NGS panel. While cell pellets had higher tumor DNA yields, supernatants can also be used for NGS. Benign supernatants have in some cases cfDNA in quantities sufficient for detection of occult neoplastic populations by NGS. Next, we aim to sequence supernatant samples with sufficient DNA yields to compare neoplastic cell genotypes with known neoplasm genetic profiles.

325 Cytologic Features of Undifferentiated and Dedifferentiated Carcinomas of the Endometrium

Amir-Hossein Akbari[1], Lu Wang[2], Robert Soslow[2], Rajmohan Murali[2]
[1]University Health Network, Toronto, ON, [2]Memorial Sloan Kettering Cancer Center, New York, NY

Disclosures: Amir-Hossein Akbari: None; Lu Wang: None; Robert Soslow: *Speaker*, Ebix/Oakstone; Rajmohan Murali: None

Background: Undifferentiated carcinomas (UCs) of the endometrium are often monomorphic solid-pattern tumors lacking overt morphologic evidence of epithelial differentiation. They are composed of small to intermediate-sized cells arranged in sheets, and frequently exhibit a characteristic dyscohesive pattern, which raises a differential diagnosis of lymphoma, plasmacytoma, high-grade endometrial stromal sarcoma or small cell carcinoma. Approximately 40% of UCs are associated with a component of (typically) low-grade endometrioid carcinoma; these cases are termed dedifferentiated carcinomas (DCs). UCs and DCs are clinically aggressive malignancies which have only become widely recognized in the past decade. We sought to describe the morphologic features of UC/DC in cytologic specimens.

Design: Cytologic specimens from patients with histologically confirmed primary endometrial UC or DC were retrieved from departmental archives. The cytologic slides were reviewed and the morphologic features listed in the Table were evaluated.

Results: Cytologic specimens from 23 women (aged 46-86 y, median 59 y) included: cervicovaginal specimens (7), peritoneal washings (5), touch preparations of core biopsies from various sites (5), fine needle biopsies of lymph nodes (3), ascitic fluid (1), pleural fluid (1) and intra-uterine fluid (1). Cytologic slides were generally cellular, although tumor cell content varied between cases. Tumor cells were singly dispersed and arranged in 3-dimensional, crowded groups (including rare papilliform structures) and exhibited high nuclear:cytoplasmic (N:C) ratios. Nuclei were predominantly round and less often oval in shape. Nuclear contours varied from smooth to irregular, and nuclear molding was not infrequent. Nucleoli were often inconspicuous. Mitotic figures and apoptotic bodies were seen in a minority of cases, and necrosis was identified in almost half. See Table for detailed cytologic features of UCs and DCs.

Cytologic features of undifferentiated and dedifferentiated carcinomas of the endometrium

		Undifferentiated carcinoma (n=12)		Dedifferentiated carcinoma (n=11)	
Parameter	**Level**	**N**	**%**	**N**	**%**
Cell arrangements	3-dimensional groups	10	83.3%	9	81.8%
	Single cells	11	91.7%	10	90.9%
	Papilliform structures	2	16.7%	2	18.2%
Cytoplasmic volume	Very scant	6	50.0%	4	36.4%
	Low	4	33.3%	3	27.3%
	Moderate	2	16.7%	4	36.4%
Nuclear shape	Round	10	83.3%	7	63.6%
	Oval	1	8.3%	1	9.1%
	Round and oval	1	8.3%	3	27.3%
Nuclear contours	Smooth	6	50.0%	2	18.2%
	Irregular	6	50.0%	9	81.8%
Nuclear molding		3	25.0%	5	45.5%
Nuclear chromatin	Coarsely granular	7	58.3%	8	72.7%
	Finely granular/salt & pepper	5	41.7%	3	27.3%
Nucleoli	Inconspicuous	6	50.0%	8	72.7%
	Single	2	16.7%	3	27.3%
	Multiple	4	33.3%	0	0.0%
Mitotic figures		3	25.0%	1	9.1%
Apoptotic bodies		5	41.7%	3	27.3%
Necrosis		5	41.7%	5	45.5%

Conclusions: UCs and DCs show a spectrum of cytomorphologic characteristics, awareness of which is important to ensure their inclusion in the differential diagnosis of cytologic specimens exhibiting these features. The presence of a relatively uniform population of (often) singly dispersed malignant cells with high N:C ratios and inconspicuous nucleoli should include UC/DC in the differential diagnosis, along with the other entities listed above. Correlation with the clinical and radiologic findings, and judicious use of ancillary tests should yield the correct diagnosis.

326 Prevalence of High-Risk HPV and Squamous Lesions in Concurrent Anal and Cervical Cytology Specimens from the Same HIV Infected Women

Majd Al Shaarani[1], Dan Lu[2], Arnold Szporn[1], Maureen Zakowski[3], Qiusheng Si[1]
[1]Icahn School of Medicine at Mount Sinai, New York, NY, [2]Mount Sinai Hospital, New York, NY, [3]Mount Sinai Medical Center, New York, NY

Disclosures: Majd Al Shaarani: None; Dan Lu: None; Arnold Szporn: None; Maureen Zakowski: None; Qiusheng Si: None

Background: HIV-infected women are at high risk for cervical and anal squamous carcinoma. Cervical and anal cytology and HPV testing has been recommended as an initial screening method for high-risk groups. This study was to investigate the prevalence of HPV genotyping and cryptologic atypia of anus and cervix from the same HIV infected women.

Design: Between 7/2012 and 7/2015, 335 HIV positive women (age 20-81,average 48.79 years,), had concurrent (within 6 months) anal and cervical cytology screening and hrHPV genotyping (cobas® HPV Test, Roche). Positive hrHPV results were reported as 16, 18 and Other (OhrHPV)) (31/33/35/39/45/51/52/56/58/59/66/68).

Results: In 335 patients, the abnormal cytology (ASCUS and up) was 65.4% (219/335) in anal specimens and 25.1% (84/335, p<0.001) in cervical specimens. Of 251 patients with negative cervical cytology, 150 (59.76%) had abnormal anal cytology and of 84 abnormal cervical cytology, 69 (82.14%) patients had abnormal anal cytology. In contrast, of 116 patients with negative anal cytology, 15 (12.93%) patients had abnormal cervical cytology and of 219 patients with abnormal anal cytology, 69 (31.50%) patients had abnormal cervical cytology. HPV was detected in 223 (66.57%) anal specimens and in 100 (29.85%) cervical specimens in the same women. The HPV genotypes are similar in the two sites (anal: HPV-16, 23.3%, HPV-18, 26.01% and HPV-other 91.03%; cervical: HPV-16, 16%, HPV-18, 21% and HPV-other 93%). 130 Of 235 (53.32%) patients with negative cervical HPV were positive for anal HPV. In 93 patients with both anal and cervical positive HPV, 43 (46%) patients had the same HPV genotypes. HPV co-infection (16 and/or 18 with other) were more commonly seen in anal specimens (77/235, 35.42%) than in cervical specimens (15/100, 15%) and co-infection was more commonly associated with high grade anal lesions (13/17, 76.47% of ASC-H+ HSIL, vs 56/161, 34.16% of ASCUS+LSIL), but it was not observed in the cervical specimens (1/4, 25% of ASH-C+HSIL vs. 10/56, 17.86% of ASCUS+LSIL).

Conclusions: (1) In the same HIV infected women, abnormal cytology and hrHPV infection are more commonly detected in the anus than in the cervix. (2) Patients with abnormal cervical cytology have higher risk of abnormal anal cytology. (3) Although HPV genotypes are similar in both sites, with OhrHPVs being the most common genotypes detected, co-infection of HPV is more commonly seen in anal specimens and more closely associated with high grade anal squamous lesion.

327 Incorporating Cytologic Adequacy Assessment into Precision Oncology Workflow using TelePathology: An Institutional Experience

Wael Al Zoughbi[1], David Kim[2], Susan Alperstein[3], Kentaro Ohara[4], Noah Greco[5], Jyothi Manohar[4], Francesca Khani[4], Brian Robinson[4], Rema Rao[2], Olivier Elemento[6], Juan Miguel Mosquera[4], Momin Siddiqui[2]
[1]Weill Cornell Medicine, New York City, NY, [2]New York-Presbyterian/Weill Cornell Medical Center, New York, NY, [3]Weill Cornell Medicine, Fishkill, NY, [4]Weill Cornell Medicine, New York, NY, [5]Weill Cornell Medical Center, New York, NY, [6]New York, NY

Disclosures: Wael Al Zoughbi: None; David Kim: None; Susan Alperstein: None; Kentaro Ohara: None; Noah Greco: None; Jyothi Manohar: None; Francesca Khani: None; Brian Robinson: None; Rema Rao: None; Olivier Elemento: None; Juan Miguel Mosquera: None; Momin Siddiqui: None

Background: Quality and quantity of a cancer patient sample is the rate-limiting factor for molecular profiling of tumors. Thus, we incorporated Rapid On-Site Evaluation (ROSE) into the workflow of precision oncology at our institution. Here, we describe our experience in adequacy assessment via Telepathology to obtain optimal samples from (68) patients with metastasis.

Design: On-Site Fine Needle Aspiration Samples (FNA) Cytology was performed for every patient who underwent a minimally invasive interventional radiology procedure requiring precision oncology. A Cytopathologist performed ROSE on the FNA samples via Telepathology, and subsequently, core needle biopsy samples were obtained for precision oncology. Fresh tissue samples were collected in real-time and submitted for biobanking. Representative frozen sections were evaluated and annotated by surgical Pathologist for nucleic acid extraction. In a retrospective manner, we evaluated the concordance between adequacy assessment and success rate of the procedure to obtain sufficient genetic martial for massively parallel sequencing (MPS).

Results: The three most common metastatic sites were: Liver (22), lymph nodes (19), and bone (18) from pan-cancer primary sites. Sixty-one procedures (89.7%) were successful to obtain optimal material to establish pathologic diagnosis—a first priority. MPS was achieved in 48 (70.6%) samples: whole exome sequencing (19); or targeted panel (28).

Adequacy evaluation predicted success rate of molecular profiling in 40/43 (93%) procedures (95% CI [80.9-98.5]). MPS was possible on 8/25 cases assessed as inadequate. All procedures (7) which ultimately failed to provide quality material for genetic analysis nor for pathological diagnosis were evaluated as inadequate on ROSE. Cell block provided sufficient DNA for MPS in (9) cases: Targeted panel (7); or WES (2). In two of these nine procedures, a core biopsy for precision oncology could not safely be performed hence the FNA martial confirmed the diagnosis and the cell blocks were used for MPS emphasizes the importance of integrating ROSE in precision medicine workflow.

Conclusions: These results support our decision to incorporate ROSE into the workflow of Precision Medicine to obtain high-quality tissue samples from metastatic lesions. In addition, molecular testing of concurrent cytology specimens with adequate cellularity is a surrogate for tissue testing if the latter failed

328 Acinar Cell Enzyme-Related Autolysis is a Frequent Occurrence in CytoLyt®-Fixed Pancreatic Fine Needle Aspiration (FNAs): An Analysis of 153 Cytologic Samples

Yazeed Alwelaie[1], Kimberly Point du Jour[1], Sonal Pandya[2], Barbara Centeno[3], Michelle Reid[1]
[1]Emory University Hospital, Atlanta, GA, [2]Emory University Hospital, Stone Mountain, GA, [3]Moffitt Cancer Center, Tampa, FL

Disclosures: Yazeed Alwelaie: None; Kimberly Point du Jour: None; Sonal Pandya: None; Barbara Centeno: None; Michelle Reid: None

Background: Pancreatic FNA material is collected in 10% formalin or CytoLyt® for cell blocks (CBs) which are critical for diagnosis and/or ancillary studies. While formalin is a standardized preservative in these samples, the effect of CytoLyt® on tissue preservation has not been systematically analyzed.

Design: Smears and CBs from CytoLyt®-fixed (CF-CB) pancreatic FNAs were blindly reviewed and the presence of tissue degradation (autolysis) noted in each CB. When available, immunocytochemical (ICC) stain intensity and % positivity was quantified in CF-CBs. Controls included formalin-fixed CBs (FF-CBs) from pancreatic FNAs and CF-CBs from non-pancreatic FNAs. The control group also included 4 pancreatic FNAs with matched CF-CB and FF-CBs.

Results: 62/85 (73%) CF-CBs showed partial to extensive tissue and/or tumor autolysis. This was most pronounced in acinar fragments or in tumors where background acinar cells were present (Figure). Of the 23 (27%) non-autolyzed CF-CBs 10 had no acinar cells and 7 had minute tissue fragments. Morphologic alterations identified in CF-CBs included poor cytoplasmic preservation and disintegration (Figure 2C), cytoplasmic clearing, and bright eosinophilic nucleoli creating more severe atypia even in benign epithelium. In contrast, autolysis was seen in only 2/46 (4%) of FF-CBs from pancreatic FNAs, and 2/26 (12%) CF-CBs from non-pancreatic lesions. Of 4 pancreatic FNAs with matched CF-CBs and FF-CBs, all 4 CF-CBs showed marked autolysis and none of the FF-CBs showed autolysis. ICC stains had less intensity and more non-specific background staining in CF-CBs than FF-CBs, causing interpretative ambiguity in 4/10 cases.

Results in 153 Cytology Specimens**		
	Number of cases	Cases with Autolysis (%)
CytoLyt Fixed (Pancreas FNAs)		
Non-diagnostic/Negative	35	27 (77%)
Atypical Cells	8	5 (63%)
Neuroendocrine Tumors	6	3 (50%)
Ductal Adenocarcinoma	29	22 (76%)
Others Neuroendocrine Carcinoma (n=1)* Granular Cell Tumor (n=1)* Acinar Cell Carcinoma (n=1)* Lymphoma (n=1)* Serous Cystadenoma (n=1) Non-mucinous Cyst (n=1) Rosai Dorfman Disease (n=1)*	7	5 (71%)
Total	**85**	**62 (73%)**
Control Group (n=72)**		
Formalin Fixed (Pancreas FNAs)		
Non-diagnostic/Negative	3	0
Neuroendocrine Tumors	23	1 (4%)
Ductal Adenocarcinoma	11	0
Acinar cell carcinoma	3	0
Others Neuroendocrine Carcinoma (n=1)* Solid Pseudopapillary Neoplasm (n=2)	6	1 (17%)

Serous Cystadenoma (n=2) Granular Cell Tumor (n=1)		
Total	**46**	**2 (4%)**
CytoLyt Fixed (non-Pancreatic FNAs)		
Lymph Nodes	6	0
Thyroid	3	1 (33.3%)
Liver	6	0
Bile Duct	2	1 (50%)
Lung	2	0
Salivary Glands	2	0
Others Adrenal (n=1)* Kidney (n=1) Soft Tissue (n=1) Adnexal (n=2)*	5	1 (20%)
Total	**26**	**3 (12%)**
* Denotes presence of tissue degradation. ** Includes 4 FF-CBs with matched CF-CBs.		

Immunocytochemistry Results for Selected Cases Fixed in CytoLyt			
Diagnosis/ICC Stains	Staining Intensity*	Percentage**	Comment
Acinar Cell Carcinoma			
Keratin (CK)	2	2	
Trypsin	3	3	High background
Ki67	1	1	Index <10%
Granular Cell Tumor			
S100 (CF-CB)	2	3	
S100 (Matched FF-CB)	3	4	
Lymphoma			
CD4	2	2	
CD5	3	3	
CD3	3	2	
Neuroendocrine Carcinoma			
Keratin (CK)	2	3	
Chromogranin	0	0	
Synaptophysin	1	1	Stronger staining in normal tissue
CD56	2	3	
Ki67	2	3	Index >40%
Neuroendocrine Tumor			
Synaptophysin	3	4	High background
Chromogranin	3	3	
*Intensity: 1: weak; 2: moderate; 3: strong. **Percentage: 1: 0–25%; 2: 26–50%; 3: 51–75%; 4: >75%.			

Figure 1 - 328

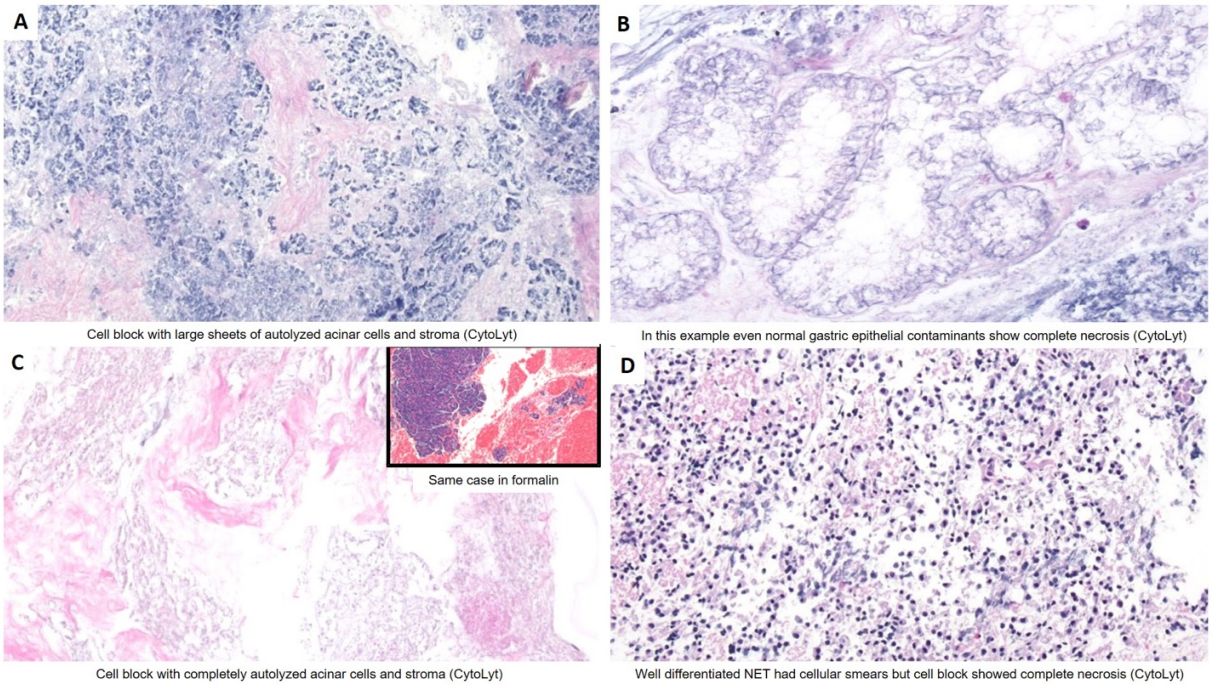
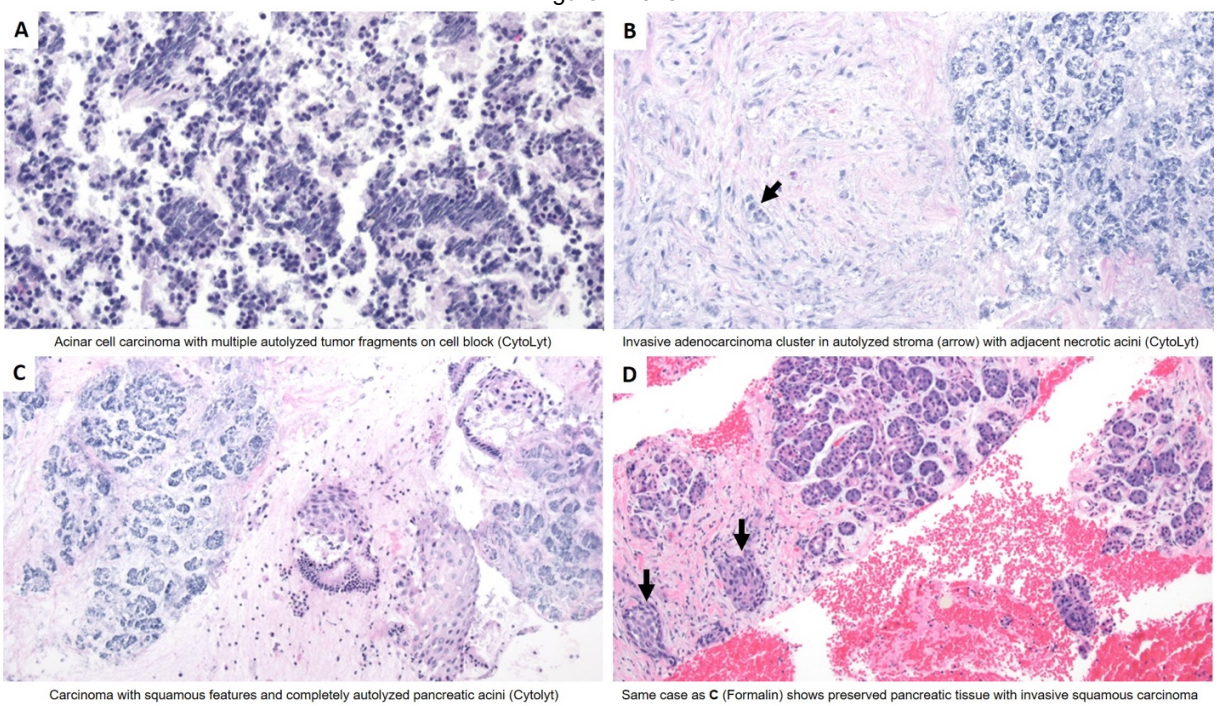


Figure 2 - 328



Conclusions: While CytoLyt® is a useful alternative to formalin for fixation of non-pancreatic FNA samples (12% rate of autolysis) it is a suboptimal fixative for fresh pancreatic FNA material and is associated with tissue/tumor autolysis in 73% of cases. Autolysis in pancreatic aspirates appears to be due to autodigestion caused by acinar enzymes, since it is most pronounced in tissues where acinar cells (both normal and neoplastic) are present. Acinar cell enzyme activity and subsequent tissue autodigestion are likely interrupted/inhibited by formalin but not by CytoLyt® in fresh pancreatic FNA specimens, thus causing accelerated tissue degradation in CF-CBs. Cytopathologists should be mindful of this limitation when using CytoLyt® fixative on fresh pancreatic FNA samples.

329 Immunocytochemical Staining Validation of Nuclear Staining Markers on Cytology Smears

Jasmeet Assi[1], Wei-Lien Billy Wang[1], Savitri Krishnamurthy[1], Yun Gong[1], John Stewart[1], Qiong Gan[1]
[1]*The University of Texas MD Anderson Cancer Center, Houston, TX*

Disclosures: Jasmeet Assi: None; Wei-Lien Billy Wang: None; Savitri Krishnamurthy: None; Yun Gong: None; John Stewart: None; Qiong Gan: None

Background: The use of immunoperoxidase staining in cytologic practice is sometimes limited by the absence or paucicellularity of the cell block preparation. As an alternative, direct smears have been used for immunocytochemical staining (ICC), but their use requires validation to document antigen stability in the preanalytic environment unique to each practice. This validation study demonstrates the reliability of using direct smears for ICC of five nuclear antigens commonly used in our practice.

Design: In this retrospective ICC validation study we tested 5 nuclear staining markers, including GATA-3, PAX-8, P40, TTF-1 and SOX-10. These markers were evaluated on Papanicolaou-stained cytology smears in ten cases with diagnoses that should have positive staining for the selected nuclear markers and also have accompanying cell-block section showing positive staining in the original case work-up for clinical diagnosis. Ten cases with known pathologic diagnoses that should not have expression of the marker were selected for each antibody as negative controls. The staining results were compared with the previously available cell block section immunostaining results and then sensitivity, specificity, negative predictive value, and positive predictive value were calculated.

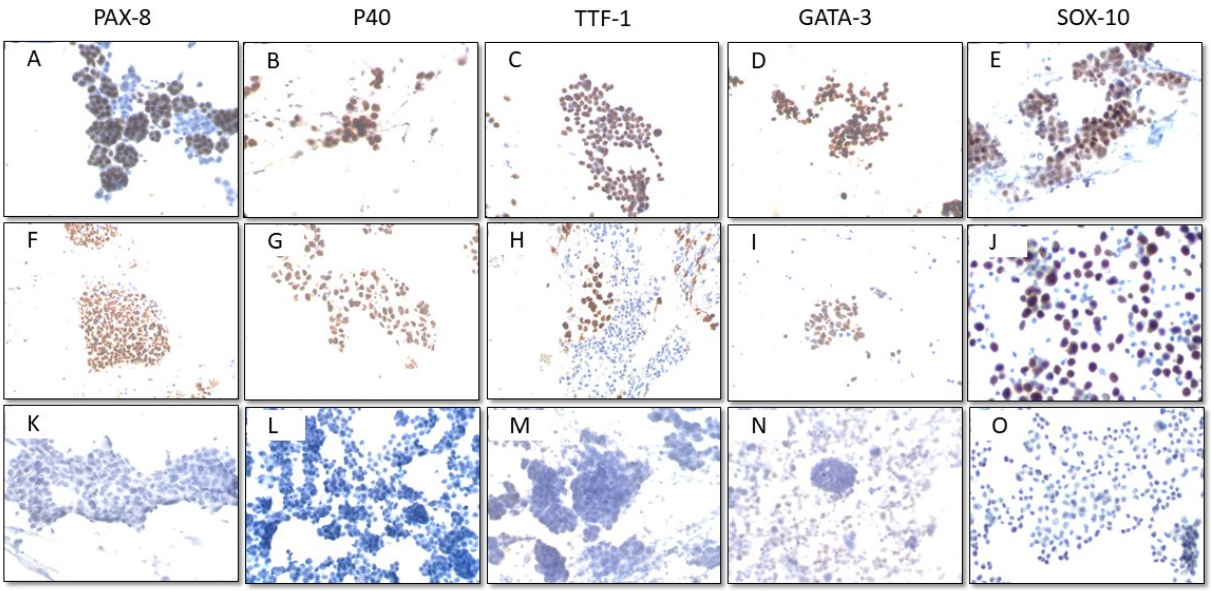
Results: ICC staining for all five nuclear antigens showed high sensitivity and specificity on direct smears (Table 1). Testing on direct smears meet recommended CAP guidelines for a diagnostic marker with >90% concordance (Figure 1). Direct smears used in this study had been stored in recommended conditions. Storage time ranged from 12 to 1141 days with mean storage times of 209-250 days. Antigen stability was demonstrate across all five antibodies tested (Table 1).

Table 1.

IHC Stain	Positive/negative specimens (n)	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	Range (days)	Mean (days)
P40	10/10	100	100	100	100	8-784	250
GATA-3	10/10	100	100	100	100	12-592	231
PAX-8	10/10	100	100	100	100	12-591	229
SOX-10	10/10	100	90	91	100	13-1141	227
TTF-1	9/10	100	100	100	100	14-469	209

Figure 1 - 329

Figure 1. Representative images of five immunostainings on smears (A-E), cell block sections (F-J), and negative smear controls (K-O)



Conclusions: 1. ICC for these five nuclear antigens using Papanicolaou-stained direct smears can be reliably used in a clinical laboratory with the same platform and staining protocols as those used for formalin -paraffin-embedded (FFPE) slides. 2. For these five antibodies, antigens on direct smears are stable and can be reliably detected up to 1141 days.

330 Yolov3 Pap Test: The Use of Publically Available Neural Networks for Screening Pap Test

Seifeldin Awad[1], Ahmad Alkashash[1], Huma Fatima[1], Isam Eltoum[1]
[1]The University of Alabama at Birmingham, Birmingham, AL

Disclosures: Huma Fatima: None; Isam Eltoum: None

Background: Increase in computer power and the release of advanced conventional neural network (CNN) algorithms made it easy for pathologists with some skill in computer programming to experiment with artificial intelligence applications . Most of publicly available training CNN image sets, however, lack cytolo/histological representative images. The objective of this study is 1) to build a large annotated image data-set for ThinPrep Pap. 2) to test a novel publicly available algorithm (You Look on Once, Yolo) on this set and assess its precision in the detection of squamous i

Design: Eleven ThinPrep tests (6 with LGSIL, 5 HGSIL, all confirmed histologically) were randomly selected. Slides were imaged using Aperio System. Whole slide image (WSI) was then de-tiled using QuPath-0.2.0. An experienced pathologist reviewed each tile and marked abnormal cells using Yolo BBox Tool. Two research fellows, then marked all normal cells. A second pathologist assessed accuracy of labeling. If there is a disagreement, the first pathologist reviewed the tile again and the diagnosis was adjudicated . Labeled cells were then used to for transfer learning using Darknet Yolov3 neural network pre-trained conventional layers weights as described by the creator of Yolo. After training, new weights were used to validate and testing Yolov3. Validation mean average precision (True Positive/(True Positive+ False Positive). Yolo was ran on Dell computer equipped with Intel® xenon CPU @2.8 GHZ and Nvidia GeForce GTX 1050.

Results: Results: WSI of ThinPrep de-tiled into mean (SD) 2381± 32 tiles. A total of 2400 well-visualized tiles from all slides were selected randomly for generating dataset. The set consisted of 50,300 individual cells with a mean 21 cell per tile. There were 41, 472 NILM, 5512 LGSIL and 3316 HGSIL cells, figure. Manual cell labeling took approximately 20 man-hrs., distributed over five months. Yolov3 algorithm took around 72 to train. Using custom weight the mean average precision ranged from 0.50 to 0.54 depending on the cut-off probability of correct identification.

Conclusions: we were able to develop the largest set of annotated ThinPrep Pap test tiles and single cells that can be used for training and validation of publically available artificial intelligence tools. Yolov3 performance under our currently setting performed poorly. We are

currently modifying the different parameters of Yolov3 and comparing its performance to other identification and detection network such as R-CNN

331 Prospective Study Evaluating the Role Of UroVysion (Fluorescence In Situ Hybridization) Assay in the Diagnosis of Upper Tract Urothelial Carcinomas (UTUC) Combined with the Paris System for Reporting Urine Cytology

Ayesha Baig[1], Fadi Brimo[2], Josee Lavoie[3], Miriam Blumenkrantz[4], Yonca Kanber[3], Derin Caglar[5], Wassim Kassouf[6], Sero Andonian[5], Manon Auger[7]
[1]McGill University, Montréal, QC, [2]McGill University, Montreal, QC, [3]McGill University Health Center, Montreal, QC, [4]Montreal Children's Hospital, Montreal, PQ, [5]McGill University Health Center and McGill University, Montreal, QC, [6]McGill University Health Centre, Montreal, QC, [7]McGill University Health Center, Mount Royal, QC

Disclosures: Ayesha Baig: None; Fadi Brimo: None; Josee Lavoie: None; Miriam Blumenkrantz: None; Yonca Kanber: None; Derin Caglar: None; Wassim Kassouf: None; Sero Andonian: None; Auger Manon: None

Background: Accurate diagnosis and management of upper tract urothelial carcinomas (UTUC) is challenging at the clinical and histological levels. We aimed to evaluate the performance of the Paris system in detecting recurrence of UTUC and the potential utility of UroVysion (FISH) in that context in a prospectively conducted study.

Design: Consecutive patients previously managed with endoscopic resection for UTUC were enrolled in their first follow-up visit. Ureter-selected washings were collected by urologist and samples for cytology and FISH were submitted concurrently. Cytology and FISH analysis were reported blindly by cytopathologists and cytogeneticists respectively. Cytological diagnoses and FISH results were correlated with the corresponding histologic follow-up.

Results: 37 samples were collected from 13 patients. The histological follow-up was benign in 15 (40%), low grade urothelial carcinoma (LGUC) in 11 (30%), and high-grade urothelial carcinoma (HGUC) in 11 (40%) cases. Cytology results: The positive predictive value (PPV) for a histological diagnosis of HGUC was 0%, 0%, and 84.6% for the cytological categories of 'negative for high grade urothelial carcinoma (NHGUC), 'atypical urothelial cells (AUC)' and 'suspicious/positive for HGUC', respectively. In comparison, the PPV for a histological diagnosis of LGUC was 11.1% for a cytological diagnosis of NHGUC, 53.3% for AUCs and 7.7% for suspicious/positive for HGUC. FISH results: FISH was positive in 26 samples (70%), of which 11 were HGUC and 9 were LGUC on histology. Overall, the false positive rate of FISH was 23% in comparison to 7% for the Paris System. Out of the 15 AUCs, 11 (73%) were positive by FISH, of which 7/11 (64%) were true-positive. In comparison, an AUC diagnosis with a FISH-negative result was associated with subsequent carcinoma in only 25% of cases.

Conclusions: The Paris System for reporting urinary cytology showed strong association with urothelial carcinoma of the upper tract, especially HGUC. Combining FISH with cytology did not improve the detection of HGUC. FISH did improve the performance of the AUC cytology category to some extent. The routine use of FISH in upper tract lesions is not justifiable.

332 Risk of Malignancy in Pancreatobiliary Cytology Categories Based on Guidelines from Papanicolaou Society of Cytopathology

Mona Bansal[1], Thom Smilari[1], Irma Oliva[2], Gretchen Galliano[1], Melissa Hamilton[1], Shams K Halat MD[3]
[1]Ochsner Health System, New Orleans, LA, [2]Ochsner Clinic Foundation, New Orleans, LA, [3]Tulane University, New Orleans, LA

Disclosures: Mona Bansal: None; Gretchen Galliano: None; Melissa Hamilton: None; Shams K Halat MD: None

Background: Endoscopic ultrasound (EUS) guided retrieval of the cytology specimen is currently the mainstay of diagnosis of pancreatic lesions due to its high accuracy. Recently Papanicolaou society of cytopathology (PSC) has released guidelines for the standardized terminology of pancreatobiliary cytology. Very studies have mentioned risk of malignancy associated with every category. Objective of our study is to evaluate risk of malignancy in our community based large cancer center in the gulf south.

Design: From Jan 2016 to Sept 2019, all pancreatic resection cases were retrieved from our pathology database. Of these, cases with corresponding cytology were selected. The cytological diagnostic categories were similar to guidelines from Papanicolaou society of cytopathology as; Non-diagnostic, negative, atypical, neoplastic, suspicious and malignant. Risk of malignancy in each category was calculated.

Results: A total of 288 patients underwent pancreatic resections. Of these, 138 had corresponding cytology with diagnosis as: Non-diagnostic-3, negative-27, atypical-3, neoplastic-27, suspicious-5, malignant-73. On surgical resection specimens, risk of malignancy was 0%, 7.4%, 33.3%, 7.4%, 100% and 100% respectively in each category. Please see attached table. For further calculations, cytology diagnosis of neoplastic, suspicious and malignancy were categorized as positive, whereas non-diagnostic, negative and atypical were lumped as negative. Statistical analysis yielded sensitivity of 81.4%, specificity-100%, NPV-27.3%, PPV-100% and accuracy of 82.6%.

CYTOLOGY		HISTOLOGY			
PSC CATEGORIES	NO. OF CASES	BENIGN	NEOPLASM	MALIGNANCY	RISK OF MALIGNANCY
NON-DIAGNOSTIC	3	0	3	0	0/3 (0%)
NEGATIVE	27	8	17	2	2/27 (7.4%)
ATYPICAL	3	1	1	1	1/3 (33.3%)
NEOPLASM	27	0	25	2	2/27 (7.4%)
SUSPICIOUS	5	0	0	5	5/5 (100%)
MALIGNANT	73	0	0	73	73/73 (100%)

Conclusions: Our study shows 100% risk of malignancy in suspicious/malignant category in our population, which is similar to published incidence. In our study, we have very few non-diagnostic (3) and atypical cases (3), partly because our practice involves EUS-cytology of mainly solid pancreatic lesions, hence our incidence may differ. Most of the cases in our neoplastic category belonged to the well-differentiated neuroendocrine neoplasms, which traditionally are considered as "not malignant".

333 MiR-10a-5p Promotes Proliferation and Development of Non-small Cell Lung Cancer by Targeting GATA6

Min Bao, Anhui Medical College, Hefei, Anhui, China

Disclosures: Min Bao: None

Background: Aberrant expression of microRNAs (miRNAs) is involved in human cancers, including non-small cell lung cancer (NSCLC). Accumulating evidence suggests that miR-10a-5p plays important roles in human carcinogenesis. However, its precise biological role remains largely elusive. This study examined the role of miR-10a-5p in NSCLC.

Design: A total of 20 pairs of tissues and 80 serum samples were obtained from NSCLC patients. Seventy-five serum samples from healthy individuals of the same age and gender were also collected. The expression of miR-10a-5p was analyzed by real-time quantitative PCR (qRT-PCR).

Lentivirus infected human lung cancer A549, and then the miR-10a-5p expression was down-regulated. The proliferation and migration, cell cycle, and apoptosis of A549 cells were detected by CCK-8, flow cytometry, and Transwell chamber method. The effect of miR-10a-5p on tumor growth was examined in nude mice xenografts as well.

The dual luciferase reporter gene assay verified whether miR-10a-5p has a direct targeted regulatory effect on the target gene. The expression of target gene in lung cancer tissues and adjacent normal tissues was analyzed by immunohistochemistry. Western blotting and qRT-PCR technique were used to detect the effect of miR-10a-5p on the protein and mRNA expression of target genes and the downstream genes.

Results: The expression levels of miRNA-10a-5p in NSCLC tissues were significantly higher than those in adjacent normal tissues. Compared with healthy controls, the miR-10a-5p in serum was overexpressed in NSCLC patients.
Down-regulation of miR-10a-5p expression in A549 cell could inhibit its proliferation, induce apoptosis, and inhibit cell invasion and migration. It could significantly reduce the tumor volume in nude mice of lung cancer xenografts.
The dual luciferase reporter assay showed miR-10a-5p is able to directly target the regulation of GATA6 Gene. the expression of GATA6 in lung cancer tissues was lower than that in adjacent normal tissues. The GATA6 expression in transplanted tumors of nude

Figure 1 - 333

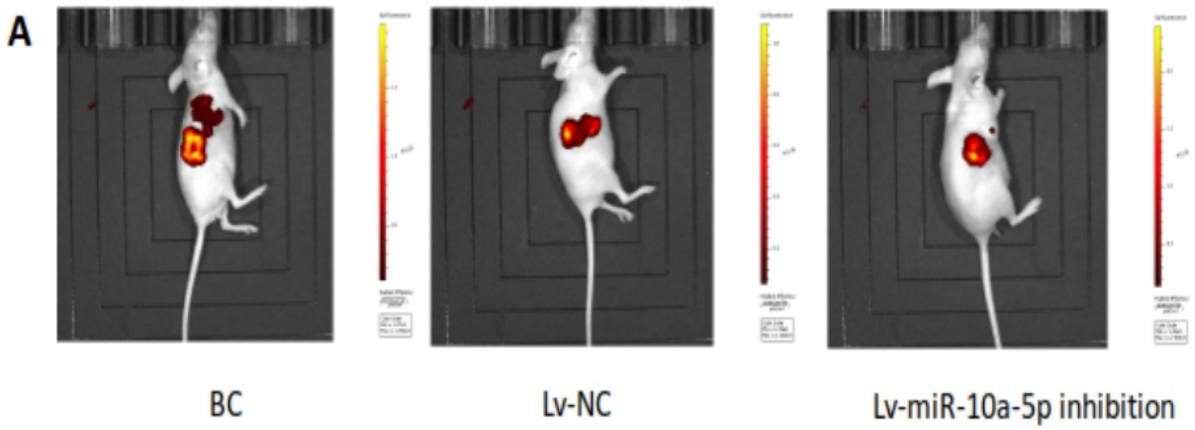
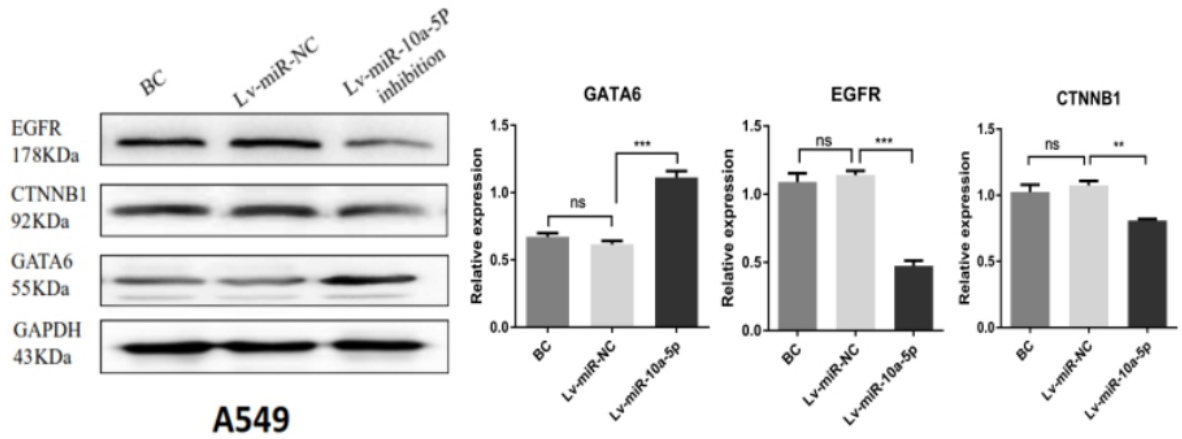


Figure 2 - 333



Conclusions: Serum miR-10a-5p may be useful biomarkers for clinical diagnosis of NSCLC.Down-regulation its expression could inhibit the proliferation, invasion, migration of A549 cells. GATA6 may be a direct target gene of miR-10a-5p, and the pathways are associated with EGFR and Wnt signals pathway.

334 Diagnostic Value of HSP70, Glypican 3 and Glutamine Synthetase in Fine-Needle Aspiration Cytology (FNAC) of Hepatocellular Nodules

Amparo Benito[1], Esther Moreno[2], Irene Carretero del Barrio[2], Cristian Perna[2], Jose Palacios Calvo[2]
[1]Hospital Ramon y Cajal, Madrid, Spain, [2]Hospital Universitario Ramón y Cajal, Madrid, Spain

Disclosures: Amparo Benito: None; Esther Moreno: None; Irene Carretero del Barrio: None; Cristian Perna: None; Jose Palacios Calvo: None

Background: Hepatic FNAC is still a useful diagnostic method for hepatocellular lesions poorly categorized by dynamic imaging techniques. Its interpretation is very complex, especially in well differentiated lesions, since it is mainly based on morphological criteria. In recent years immunohistochemistry in liver histologic material has been proved very useful for discriminating benign from malignant hepatocellular nodules. However, to our knowledge immunocytochemistry (ICC) has not yet been fully validated in FNAC samples.

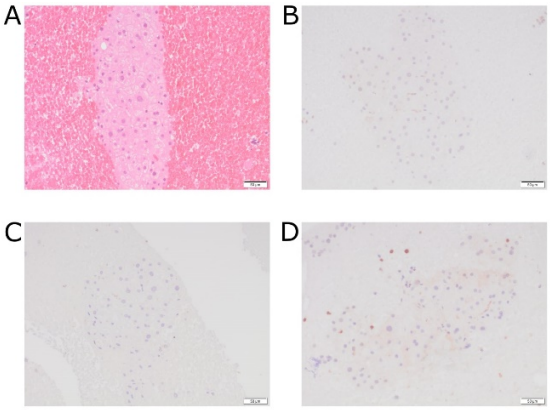
Design: Thirty-four archival, paraffin-embedded FNAC cell blocks with enough material representing hepatocellular nodules (11 benign and 23 malignant) were selected. They were immunostained with HSP70, Glypican 3 (GPC-3) and Glutamine Synthetase (GS). Clinical follow-up and further histological studies of the patients were reviewed.

Results: The sensitivity and specificity for hepatocellular carcinoma (HCC) diagnosis were 91.3% and 72.7% for GS, 91.3% and 81.8% for HSP70, and 52.2% and 100% for GPC-3; respectively. In the benign group, none of the archival cases stained for GPC-3. Only one (9.1%) stained positively (weak and focal staining) for 2 markers (GS and HSP70). In the HCC group, 21 (91.3%) stained positively for at least 2 of

the 3 markers used. Applying this panel, 5 of the archival cases morphologically diagnosed initially as benign or atypical in FNAC would have been correctly diagnosed as malignant. All these 5 cases had had a subsequent histological diagnosis of HCC.

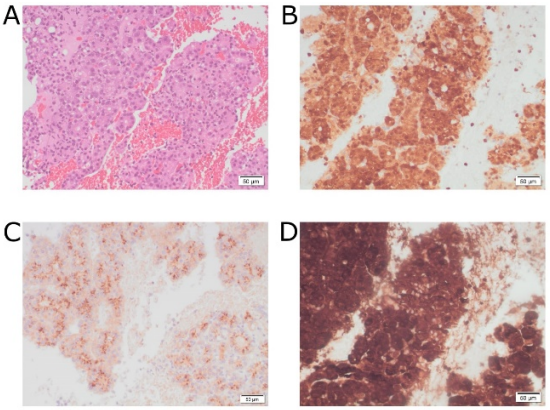
	TOTAL	GS +	HSP70 +	GPC-3 +	2 OR 3 +
	Count (%)				
BENIGN	11 (100)	3 (27.2)	2 (18.1)	0 (0)	1 (9.1)
HCC	23 (100)	21 (87.5)	21 (87.5)	12 (52.2)	21 (91.3)

Figure 1 - 334



Benign hepatocellular nodule. **A:** HE. **B:** HSP70. **C:** Glypican 3. **D:** Glutamine sintetasa.

Figure 2 - 334



Hepatocellular carcinoma grade 2. **A:** HE. **B:** HSP70. **C:** Glypican 3. **D:** Glutamine sintetasa.

Conclusions: High grade hepatocellular lesions are correctly categorized as malignant by cytological criteria. In well differentiated tumors, the proposed ICC panel yields better diagnostic accuracy than conventional cytological analysis.

335 Morphologic Patterns of Prostate Cancer Metastases to the Lung in Cytology and Histology: A Review of 30 Cases

Alexander Blank[1], Roni Cox[2], Erika Doxtader[3], Lanisha Fuller[4], Jesse McKenney[4], Sanjay Mukhopadhyay[4], Christopher Przybycin[4], Scott Robertson[4], Jordan Reynolds[4], Jane Nguyen[4]
[1]Cleveland Clinic Pathology and Laboratory Medicine, Cleveland Heights, OH, [2]Cleveland, OH, [3]Cleveland Clinic, Pepper Pike, OH, [4]Cleveland Clinic, Cleveland, OH

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Background: Prostatic adenocarcinoma (PrCA) with metastases to the lung and mediastinal lymph nodes is uncommon. This study reviews a series of such cases, with cytological, histological and immunohistochemical correlation.

Design: After approval from our institutional IRB, patients with metastatic PrCA to the lung or mediastinal lymph nodes from 2015 to 2019 were identified and reviewed for morphologic analysis. Cytologic and histologic specimens were included.

Results: A total of 30 cases were identified including 18 cytology specimens and 12 biopsies (2 wedge-, 7 lung needle - and 3 lymph node biopsies). Sites included lung, mediastinal, subcarinal and hilar lymph nodes. Four morphological patterns were identified. Cytology cases most commonly showed a solid sheet pattern (10/18, 55.6%), cribriform pattern (7/18, 38.9%) with a single case of mixed cribriform/solid sheet pattern (1/18 5.6%). No cases showed well-formed acinar formation. The most commonly noted pattern on biopsy specimens was cribriform (7/12, 58.3%), followed by solid sheet pattern (2/12, 16.7%), mixed cribriform/solid sheet pattern (1/12, 8.3%), and ductal pattern (1/12, 8.3%). A single case of tubulopapillary pattern was identified (8.3%). Immunohistochemical staining was performed in 14/18 cytology cases. While NKX3.1 was positive in all cases (13/13, 100%), Prostate specific antigen (PSA) was positive in 5/9 cytology cases (55.6%). Both NKX3.1 and PSA were positive in all surgical cases (performed in 12 and 6 cases, respectively). Gleason scores of previous prostate specimens were available in 17 cases, either through previous departmental reports or by clinical notes in the electronic medical record system. Gleason scores ranged from 6 (2 cases) to 9. Both cases with Gleason score 6 were signed out outside of our institution 13 and 18 years ago with sparse additional information (time from initial diagnosis to metastasis were 10 and 17 years, respectively) found in clinical notes. If reviewed today, it could not be excluded that there were some areas of higher grade missed or not sampled.

Conclusions: Metastatic PrCA to the thoracic cavity presented in high-grade patterns (cribriform and solid sheets) with no "well-differentiated" patterns identified. A broad panel of stains should be used to confirm metastatic PrCA to the lung, as single stains may be negative in this setting.

336 HPV Primary Cervical Cancer Screening in Portugal (Setubal Health Region)

Maria Jose Brito[1], Catrine Dahlstedt-Ferreira[2], Ana Alves[3], Eunice Carrapiço[4], Ana Botica-Quintas[2]
[1]Hospital Garcia de Orta, Lisboa, Portugal, [2]Hospital Garcia de Orta, Almada, Portugal, [3]Feijó, Almada, Portugal, [4]ARSLVT, Lisboa, Portugal

Disclosures: Maria Jose Brito: None; Catrine Dahlstedt-Ferreira: None; Ana Alves: None; Eunice Carrapiço: None; Ana Botica-Quintas: None

Background: Portugal's National Cervical Screening Program currently recommends HPV primary screening every 5 years for women aged 30-65 years. Human papillomavirus (HPV) vaccination was implemented in 2008 with 86% population coverage. But for women that have not been vaccinated, mainly the ones that are now older than 23 years old, a high risk of developing cervical cancer remains. In the Lisbon-Tagus River Valley Region, the HPV Primary Screening was implemented during 2018.

Design: We analysed the 18704 HPV Primary Screening tests performed in the Setubal Peninsula Region (southern Portugal) over a period of 1.5 years after implementation of the HPV primary screening, the test results, the number of colposcopy referrals and the type of lesions found. Women aged 30-65 years, where evenly distributed in the different age decades. The HPV Primary Test used was a mRNA test (APTIMA®-Hologic) carried out in liquid-based cytology specimens (Thin Prep®-Hologic). According to the HPV Primary Test results patients were immediately referred to colposcopy (if HPV 16 or 18/45 positive) or, if positive for any of the other pooled HPV high risk (31, 33, 35, 51, 52, 56, 58, 59, 66, and 68) had a complementary cytology exam and if any alteration was found (ASC-US, ASC-H, LSIL, HSIL, AGC)* women were also referred to colposcopy and biopsies** performed. Nomenclature from the *Bethesda System (2014) and the **Lower Anogenital Squamous Terminology (LAST) Project (2012) was utilized.

Results: 18704 HPV tests and of 900 colposcopy exams were performed. Screening program adhesion: 72.92%. Global PPV (Predictive Positive Value) for high-grade squamous intra-epithelial lesions or more (HSIL+): 18.3%. PPV of HPV16+ for HSIL+: 50.7%.

Total HPV tests	18 704					
Total HPV positive	1676 (8.9%)					
Non-valid tests	26 (0.14%)					
			Colposcopic Biopsies (n=961)			
			Normal/ Inflammation	**LSIL**	**HSIL**	**ADC in situ**
HPV 16	280 (1.4%)		131(46.8%)	7 (2.5%)	142 (50.7%) (4 SCC in Cone)	1(0.04%) (1 ADC in situ in cone)
HPV 18/45	129 (0.7%)		110 (85.2%)	7 (5.4%)	11 (8.5%)	1(0.8%)
HPV Co-infection 16+18/45	13 (0.06%)		5 (39%)	1(7%)	7(54%)	0
HPV HighRisk other than 16,18/45	1255 (74%)					
Cytology	NILM	635 (50.6%)				
			Colposcopic Biopsies			
			Normal/Inflammation	**LSIL**	**HSIL**	**ADC in situ**
	ASC-US	255	93 (37%)	120 (47%)	42 (16.4%)	
	ASC-H	46	10 (22%)	0	36 (78%)	
	AGC	10	9 (90%)	0	0	1(1%)
	LSIL	241	0	241 (100%)	0	
	HSIL	68	0	0	68 (100%)	

Conclusions: This program displays a good adhesion from women invited to participate 72.92% and good PPVs. Follow up data are necessary to evaluate NPV (negative predictive value) in this population.

337 Utility of Cytologic Evaluation of Body Cavity Fluids in Pediatric Patients for First-Time Diagnosis of Malignancy and Recurrence

Stephanie Bryant[1], Kamal Khurana[2]
[1]SUNY Upstate Medical University, Syracuse, NY, [2]SUNY Health Science Center, Syracuse, NY

Disclosures: Stephanie Bryant: None; Kamal Khurana: None

Background: The utility of body cavity fluids cytology for the primary diagnosis of malignancy or recurrence is well documented in adults. However, its role in the pediatric population is less defined. We reviewed cytology of pleural, pericardial and peritoneal fluids in the pediatric age group to determine its value in first-time diagnosis of malignancy or recurrence.

Design: A cytopathology database search between January 2003 to August 2019 was performed to identify pleural, pericardial, and peritoneal fluid specimens from patients under the age of 20 years. Relevant clinical information, pathology and cytology was reviewed. The data were also analyzed for use of ancillary studies, type of malignancy, and primary diagnosis or recurrence.

Results: One hundred eighty-two body cavity fluid specimens from 151 patients were identified. Twenty-two specimens (12%) from 21 patients (range 2-18 years, mean 9 years) were positive for malignancy. The specimens were positive in 11 out of 97 pleural fluids (11%), one out of 13 pericardial fluids (8%), and 17 out of 72 peritoneal fluids (24%). Eight patients (38%) had a prior diagnosis of malignancy while initial diagnosis was made on cytologic examination in 13 patients (62%). Ancillary studies including immunohistochemistry and/or flow cytometry were used in 16 (73%) of the positive cases. Malignant specimens consisted of lymphoma/leukemia (52%), blastoma (18%), sarcoma (19%), carcinoma (5%), and germ cell tumor (5%).

Conclusions: Our observations underscore the importance of accurate cytologic evaluation of pleural, pericardial and peritoneal fluids in the pediatric population for first-time diagnosis and recurrence of malignant disease. First-time cytologic diagnosis of malignancy on body cavity fluid may obviate the need for more invasive procedure for tissue diagnosis. The overall rate of malignant effusion of 12% in children is in line with previously published rates.

338 Utility of HER2 Testing in Fine Needle Aspiration and Core Biopsy Samples of Gastrointestinal and Pancreaticobiliary Tract Malignancies

Anne Chen[1], Gregory Scott[2], Brock Martin[3]
[1]Stanford University, Stanford, CA, [2]Redwood City, CA, [3]Stanford University School of Medicine, Stanford, CA

Disclosures: Anne Chen: None; Gregory Scott: None; Brock Martin: None

Background: Fine needle aspiration (FNA), with or without core biopsy, is a common modality to acquire tissue for diagnosis and prognostic/predictive testing, particularly in the setting of locally advanced, unresectable and/or metastatic disease. With the success of HER2-targeted therapy in gastroesophageal adenocarcinomas and trials for its use in metastatic colorectal cancer, HER2 testing is increasingly requested in our cytopathology laboratory. Clinicians are also more frequently requesting HER2 testing on pancreaticobiliary tract primaries, for which there are no well-established specimen testing guidelines. Investigation into the utility of HER2 expression analysis in FNA biopsies of gastrointestinal and pancreatobiliary tract malignancies is warranted.

Design: The anatomic pathology database at a single tertiary academic medical center was searched for FNA cytology specimens with HER2 testing performed on a gastrointestinal or pancreatobiliary tract malignancy. The results of immunohistochemistry and fluorescence *in situ* hybridization (FISH) studies were recorded, and rates of HER2 positive, equivocal, and negative status were calculated separately for upper gastrointestinal tract, colorectal, and pancreaticobiliary tract primary malignancies.

Results: 115 FNA samples of gastrointestinal and pancreatobiliary tract tumors with HER2 testing were identified. In most cases, HER2 immunohistochemistry (IHC) was performed with FISH testing initiated for equivocal cases. The proportion of cases with a positive HER2 end result was 12% (5/41) in upper gastrointestinal tract primaries, 4% (2/52) in colorectal primaries, and 14% (3/22) in pancreaticobiliary primaries. Only 2.6% (3/115) of FNAs resulted in an equivocal HER2 end result.

HER2 Immunohistochemistry and FISH Results in Gastrointestinal and Pancreatobiliary Tract Malignancies Biopsied by FNA

HER2 Result	Upper Gastrointestinal (N = 41)	Colorectal (N = 52)	Pancreaticobiliary (N = 22)
Positive	5 (12%) 5 IHC Score 3+	2 (4%) 2 IHC Score 3+	3 (14%) 1 IHC Score 3+ 1 IHC Score 2+ and FISH positive 1 FISH positive (no IHC)
Equivocal	1 (2%) 1 IHC equivocal and FISH equivocal	0 (0%)	2 (9%) 1 IHC equivocal and FISH equivocal 1 IHC equivocal and insufficient sample for FISH
Negative	35 (85%) 30 IHC Score 0-1+ 4 IHC equivocal and FISH negative 1 FISH negative (no IHC)	50 (96%) 50 IHC Score 0-1+	17 (77%) 15 IHC Score 0-1+ 2 IHC equivocal and FISH negative

Conclusions: These data confirm that HER2 testing on FNA specimens of gastrointestinal and pancreaticobiliary tumors is a minimally-invasive and cost-effective modality to identify therapeutically actionable HER2 results in a subset of patients. While the rates of HER2 abnormality in this smaller study are comparable to those published in large-scale HER2 expression studies of various malignancies, caution must be exercised in the setting of negative results given previously well-documented intratumoral heterogeneity for HER2 expression in gastrointestinal carcinomas. Further investigation into standardization of testing for pancreatobiliary tract malignancies is needed, as outcome-based guidelines are currently lacking.

339 Comparing Cytopathologists' Accuracy of Subclassification and Grading of Renal Tumors on Fine Needle Aspirations

Heather Chen[1], Razvan Lapadat[2], Anna Biernacka[1], Ward Reeves[1], Jeffrey Mueller[2], Ricardo Lastra[1], Tatjana Antic[1]
[1]The University of Chicago, Chicago, IL, [2]University of Chicago Medical Center, Chicago, IL

Disclosures: Heather Chen: None; Razvan Lapadat: None; Anna Biernacka: None; Ward Reeves: None; Jeffrey Mueller: None; Ricardo Lastra: None; Tatjana Antic: None

Background: According to literature fine needle aspiration (FNA) of renal lesions can distinguish between benign from malignant lesions in 73% to 94% of cases. Older studies suggested the correct subclassification of renal cell carcinomas (RCCs) by cytomorphology can be achieved in up to 80% of cases. However, as renal cell carcinomas become increasingly subclassified by molecular signatures, it is unsure RCCs can be classified by cytopathology alone.

Design: Two FNA passes (2 Diff-Quick and 2 Pap stained smears) were performed on all fresh nephrectomy specimens for a 1-year period. The study included 30 cases representing primary renal tumors and one case of metastatic lung adenocarcinoma (Table 1). The cases were assigned a random number and 5 cytopathologists with 3 to 22 years of experience were asked to provide a diagnosis and WHO/ISUP grading if applicable based off 2 slides for each case (Diff-Quick and Pap). Each answer was assigned a numerical code (1 to 20). Fleiss' Kappa and Cohen's Kappa equations were used to look at inter-rater variability.

Results: In terms of accuracy of diagnosis, the percentage of correct diagnosis for each cytopathologist ranged from 20 to 30% for an average of 26%. For clear cell RCC (CCRCC), the accuracy improves with 29 to 64% of correct responses, for an average of 48%. Of the papillary RCCs (PRCC), accuracy decreased with only 3 of the 5 pathologists calling up to 2 cases a papillary RCC. None of the chromophobes, rarer RCCs, mesenchymal tumors or metastasis were diagnosed accurately on FNA. Between cytopathologists, the inter-rater variability for all RCC diagnoses had fair agreement with a Fleiss' Kappa coefficient of 0.24. By RCCs subtype, the best inter-rater variability value is for CCRCC with a Fleiss' Kappa coefficient of 0.29. The second-best coefficient was for the rarer RCCs with a coefficient of 0.22. The worst inter-variability number came from the chromophobe RCCs with a coefficient of 0.03. For WHO/ISUP grade,

the weighted Cohen's Kappa coefficient for each pathologist compared to the nephrectomy ranged from 0.12 to 0.47, ranging from fair to moderate. The Fleiss' Kappa for all cytopathologists was -0.04, indicating poor agreement amongst each other. However, grading was done when not applicable.

Table 1

Surgical Pathology Diagnosis	Number
Clear cell RCC (CCRCC)	14
Papillary RCC (RCC), type 1	3
Papillary RCC, type 2	3
Chromophobe	4
Oncocytoma	1
Clear cell papillary RCC	1
Sclerosing perivascular epithelioid tumor	1
Unclassified RCC with *NF2* mutation	1
Oncocytic RCC with *TSC* mutation	1
Lung metastasis	1

Conclusions: If the RCC is a common subtype, it is unlikely to be accurately classified. Even with CCRCCs, the accuracy of diagnosis is suboptimal. Amongst cytopathologists, there is fair agreement for the subtype, but poor agreement for grade.

340 Fast Formalin Fixed (Triple F) Cell Block, a Tissue Biopsy Equivalent Cell Block Augmenting Ancillary Test of Fine Needle Aspiration Biopsy

Hua Chen[1], Sharmila Ghosh[2]
[1]Icahn School of Medicine at Mount Sinai, Elmwood Park, NJ, [2]Mount Sinai West, Icahn School of Medicine, New York, NY

Disclosures: Hua Chen: None; Sharmila Ghosh: None

Background: Cell block (CB) is a very useful adjunct to fine needle aspiration biopsy (FNA). There are numerous techniques of making CB, mostly using non-formalin-based fixatives and labor intensive. Limitations of these techniques include low cellularity due to loss of cellular material during centrifugation, long hands-on preparation time requiring multiple steps, lack of validation of Immunohistochemical (IHC) stains performed on non-formalin fixed material, edge artifacts of fragmented cellular material, possible false-negative results for prognostic marker testing due to prolonged cold ischemic time or fixation in non-formalin based fixative. In this report, we develop fast, formalin fixed (Triple F) CB preparation method.

Design: Triple F CB protocol: Step 1. The target lesion was aspirated and the needle hub allowed to fill with aspirated material. Step 2. On completion of the aspiration, the needle was removed from the target. Step 3. A 10 minute wait time allowed the aspirated material to form a solid blood clot. Step 4. The blood clot was rinsed from the needle hub with 10% neutral buffered formalin (NBF) and the clot placed on filter paper. Step 5. The blood clot was submitted in NBF formalin for tissue processing. Consecutive 41 Triple F CB from January 2019 were analyzed.

Results: In total, 93% of the Triple F CBs were diagnostic. 63% of the Triple F CB had cellularity greater than 100 cells and were adequate for prognostic marker analysis. 29% of the Triple F CBs had cellularity between 10 to 100 cells adequate for diagnostic work up. Total preparation time was 15 minutes with less than 5 minutes hands-on time. No centrifuge was used. All the Triple F CBs were made at FNA clinic onsite at the time of collection. Up to 29 IHC stains could be performed on a single Triple F CB. IHC performed on Triple F CB was 100% in concordance with IHC performed on tissue specimen of the same lesion. No false negative results were identified. The background was very clean and no edge artifacts were identified. Immediate fixation with formalin was also incompliance with CAP protocol for prognostic markers analysis.

Conclusions: Triple F cell block is a tissue grade cell block with high cellularity, superior morphology and minimal edge artifact. It is easy to perform, quick and cost effective. Immediate fixation in formalin is identical to tissue biopsy processing and no additional validation is needed for immunohistochemical stain.

341 An Algorithmic Approach to Diagnose Malignant Mesothelioma in Body Effusion

Lan Chen[1], Wei Zhang[2], Jing Wang[3], Dongge Liu[1]
[1]Beijing Hospital, Beijing, China, [2]Beijing, China, [3]Beijing Key Laboratory of Head and Neck Molecular Diagnostic Pathology, Beijing Tongren Hospital, Capital Medical University, Beijing, China

Disclosures: Lan Chen: None; Wei Zhang: None; Jing Wang: None; Dongge Liu: None

Background: Cytologic diagnosis of malignant mesothelioma (MM) in body effusion is challenging because MM spans a wide spectrum from mild atypia to poor differentiation overlapping with benign mesothelial proliferation on one hand and adenocarcinoma on the other. Accurate diagnosis in body effusion brings less harm and earlier treatment to patients as compared with biopsy.

We studied morphology and immunohistochemical (IHC) phenotype on cell blocks and P16 florescence in situ hybridization (FISH) to set up an algorithmic approach in diagnosis of MM in effusion.

Design: A case-control study included 71 MM and 40 benign mesothelial hyperplasia (MH) in effusion from a single hospital's collection from 2011 to 2019. All cases were later confirmed by biopsy and/or clinical image and manifestation. Morphology analysis includes mesothelial cell characteristics, atypia, large red nucleoli, multinucleation, cannibalism and mirror ball-like structure. An IHC panel (Zhongshan, China) of CK7, CK20, D2-40, WT1, Desmin, CEA, EMA, Glut1, P53 were applied on cell blocks together with site-specific markers of TTF-1, PAX8 and CDX2 when relevant. P16 FISH was done in 17 MM using Vysisi CDKN2A/CEP 9 FISH Probe. BAP1(Santa Cruz) was also studied.

Results: Morphology analysis indicated that tumor cells were bigger, more polymorphic with more prominent red nucleoli as compared with that of MH. However, cannibalism, multinucleation and even disc ball-like structure could also be observed in MH, especially in pericardial effusion, therefore, ancillary study of IHC should play an important role in differential diagnosis.

EMA and Glut1 were expressed strongly in 87% of MM cases separately, but were hardly expressed in MH. 56.3% of MM had a strong P53 expression over 5% to 50% of tumor cells. Desmin was expressed in 96.9% of MH cases, but scantly expressed in 8.3% of MM. CEA was negative in both MM and MH. Over 80% of MM and MH were positive for WT1, D2-40 and CK7, while they were all negative for site specific markers of TTF-1, gata3 and CDX2. Loss of BAP1 was observed in over 50% of MM, but not in MH. 7 out of 17 MM had P16 homozygous deletion.

Lesion\IHC	case	CK7	CK20	D2-40	WT-1	Desmin	EMA	CEA	Glut1	P53
MM	71	+	-	+	+	+	+	-	+	+
MM%		69.6	-	87.5	87.5	8.3	87.0	-	87.0	56.3
MH	40	+	-	+	+	+	+	-	+	-
MH%		87.9	-	81.2	81.8	96.9	19.4	-	14.3	-

Table1. IHC expression in MM and MH

Figure 1 - 341

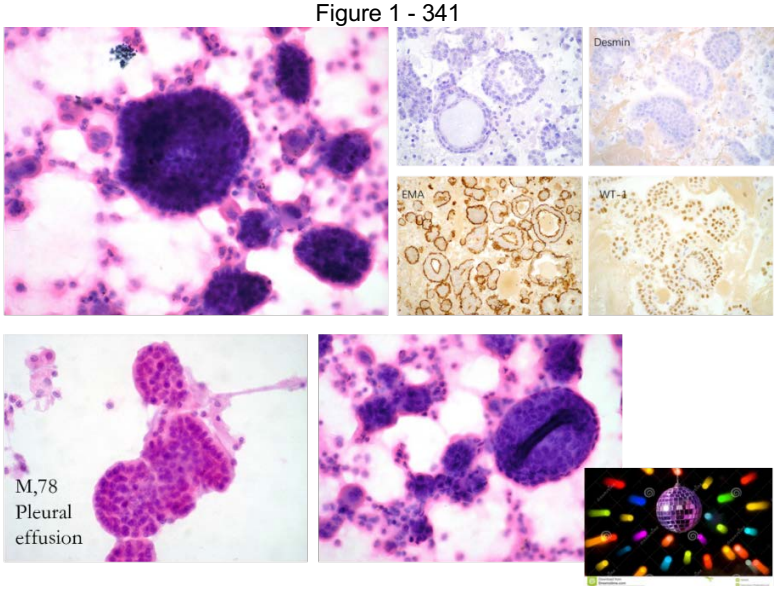
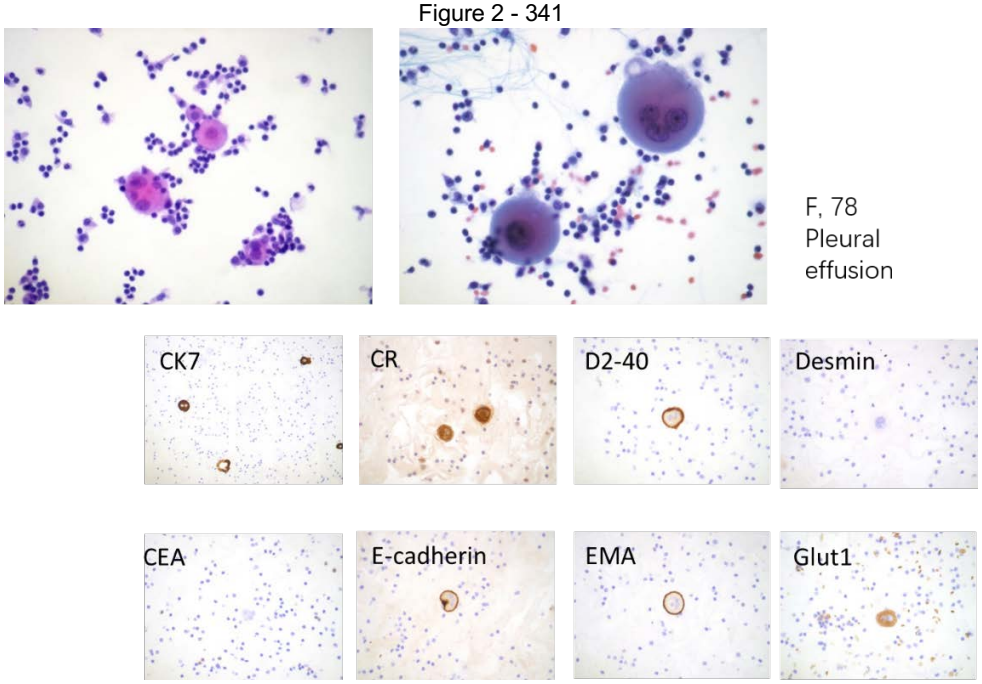


Figure 2 - 341



Conclusions: Cytology can help make an early diagnosis of MM when combined with IHC and P16 FISH. An expression pattern of CK7, CK20, D2-40 and WT-1 can indicate cell origin of targeted lesion while CEA, TTF-1, CDX2 and PAX8 can exclude site specific adenocarcinoma. BAP1, P53, Desmin, Glut1 and EMA are useful markers to differentiate reactive from malignant mesothelial cells.

342 Rapid Immunocytochemistry

Shuo Chen[1], Ziman Cheng[2], Yan Zhang[3], Jianfu Wang[2], Jin Wu[4]
[1]Novodiax I, Hayward, CA, [2]Novodiax Inc, Hayward, CA, [3]Hayward, CA, [4]Novodiax, Inc, Hayward, CA

Disclosures: Shuo Chen: *Employee*, Nonodiax Inc; Ziman Cheng: *Employee*, Novodiax Inc; Yan Zhang: *Employee*, Novodiax Inc; Jianfu Wang: *Employee*, Novodiax Inc; Jin Wu: *Employee*, Novodiax, Inc

Background: Rapid immunohistochemistry (IHC) on frozen sections can be finished in 10 minutes when using the proprietary directly conjugated polymer HRP-antibodies. Currently, 16 rapid IHC products (*IVD*) are available for intraoperative immunostaining. The objective of the present study is to examine the proper working conditions for applying some of these reagents to cell smear preparations while cells still retain their apparent integrities, achieving rapid immunocytochemistry (ICC) on fresh tissues.

Design: Rapid IHC reagents including polyHRP-Mart-1, Ki67, SOX-10, CK8/18, CK5, AE1/3, CD20, CD45, Pan-CK were examined using cell smears prepared from human cheek cells or cultured cell lines: SiHa (cervical cancer), A375 (melanoma, SOX-10 positive, Mart-1 negative), SK-Mel (melanoma, SOX-10 positive, Mart-1 positive), SK-Br3 (breast cancer), GA-10 (B lymphocytes), MJ[G11] (T lymphocytes). Cell smears were pretreated with one of the four types fixatives prior to the staining procedure: acetone, neutral buffered formalin (NBF), alcohol (40% in PBS) and Dent (80% MeOH 20% DMSO). A rapid ICC staining protocol was adopted with adjustments of incubation time, usually completing the stain within 10-20 minutes.

Results: PolyHRP-antibodies rapidly stain fresh cell smears in the similarly way to that on frozen sections but with a major difference: cells in the smear preparation are intact – whole cells. Therefore, these cells have to be pretreated before the staining procedure. The results of pretreatment are shown in the following table (Table 1). Check marks represent satisfactory staining (Examples: Fig. 1: pHRP-CD45 on T cells; Fig. 2 pHRP-CK8/18 on breast cancer cells). Otherwise indicate unacceptable stains.

ICC Pretreatment for pHRP-antibody Conjugates				
pHRP-	**Acetone**	**NBF**	**EtOH**	**Dent**
Ki67	√	√		
SOX10	√	√		
Mart-1	√	√		
CK8/18	√	√		
pan-CK	√	√		
CK5	√			
AE1/3	√	√	√	
CD 20	√			√
CD 45	√	√	√	

Figure 1 - 342

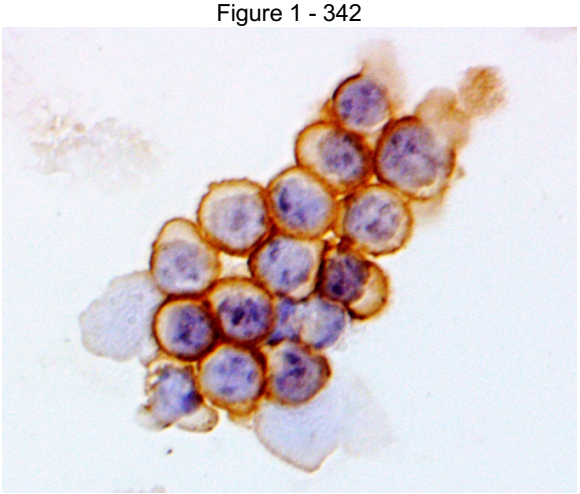
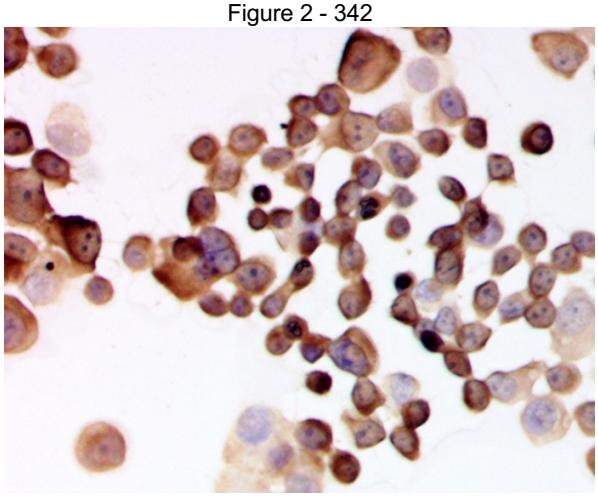


Figure 2 - 342



Conclusions: The pretreatment procedure to fresh cell smear serves for two purpose: fixation and perforation of cell membrane. Among four common fixatives we have evaluated, the acetone and NBF performed the best. In summary: with a proper pretreatment on fresh cell smears, a rapid ICC can be achieved on whole cell preparations. This suggests that these pHRP-antibody reagents can potentially be a useful tool for rapid on-site evaluation (ROSE) to resolvet targets at molecular level.

343 Comparisons of PD-L1 Expression Between Paired Cytologic and Histologic Specimens Obtained by Endobronchial Ultrasound-Guided Transbronchial Needle Aspiration in Non-Small Cell Lung Cancer Patients

Ying Chen[1], Xuxia Shen[2], Yanli Wang[1], Yuwei Zheng[1], Longfu Wang[1], Xu Cai[3], Yuan Li[4], Ping Bo[1]
[1]Fudan University Shanghai Cancer Center, and Department of Oncology, Shanghai Medical College, Fudan University, Shanghai, China, [2]Fudan University Shanghai Cancer Center; Shanghai Medical College, Fudan University, Shanghai, China, [3]Fudan University Shanghai Cancer Center, Shanghai, China, [4]Shanghai Cancer Hospital, Shanghai, China

Disclosures: Ying Chen: None; Xuxia Shen: None; Xu Cai: None

Background: PD-L1 expression is one of the predictive biomarkers for anti-PD-1 immunotherapy in NSCLC. Because the current indications are for advanced patients, that are often diagnosed by minimally invasive methods rather than surgical resection. EBUS-TBN is one of the minimally invasive techniques. In this study, we investigated if PD-L1 expression shows comparable results on paired cytologic and histologic tumor specimens obtained by EBUS-TBNA from patients with NSCLC.

Design: A total 50 cases of NSCLC diagnosed by EBUS-TBNA that had paired cytological and histological specimens were collected from August 2018 to August 2019 in our Hospital. Immunohistochemical staining were performed in each case for subtyping. Cell block sections and histological biopsy sections were stained with PD-L1 antibody (22C3) and only cell block slides with 100 or more tumor cells were evaluated. Tumor proportion scores (TPS) were assessed on the basis of partial/complete membranous staining of tumor cells. Hematoxylin staining, CD68 and AE1/AE3 staining were performed in the adjacent continuous sections of PD-L1 staining section to identify the number of tumor cells in the cell block sections. In 30 of the 50 cases, EGFR mutation, ALK, ROS-1 and MET gene amplification were also detected.

Results: Immunohistochemical results showed 34 cases adenocarcinoma, 10 cases squamous cell carcinoma and 6 cases NSCLC, not otherwise specified. For 28 of the 50 cases cell block sections evaluated of PD-L1 (56%) with TPS < 1%; 8 cases (16%) with TPS in 1% - 49%; and 14 cases (28%) with TPS > = 50%. The 50 matched histology specimens showed 28 cases (56%) with TPS < 1%; 8 cases (16%) with TPS in 1% - 49%; 13 cases (26%) with TPS >= 50%; and 1 case (2%) with not being evaluated because the number of tumor cells in the section were less than 100. The coincidence rate was 91.83% between cell blocks and histological biopsy specimens, and the Kappa value was 0.830. Only 4 cases were discordant between cell blocks and histological specimens, which was not related to the number of tumor cells in cell block sections, but likely because of intratumoral heterogeneity. There was no significant correlation between PD-L1 staining TPS and patient gender, smoking history, tumor stage, and classical lung cancer driver gene changes.

Conclusions: The results show that NSCLC EBUS-TBNA cell block samples evaluated for PD-L1 have high concordance with paired histologic biopsy samples and can be used for supplementary evaluation of PD-L1 expression.

344 Prevalence of High-Risk Human Papillomavirus Types in Unsatisfactory Anal Cytology Specimens and Correlation with Histology

Bonnie Choy[1], Amy Ly[2]
[1]Northwestern University Feinberg School of Medicine, Chicago, IL, [2]Massachusetts General Hospital, Boston, MA

Disclosures: Bonnie Choy: None; Amy Ly: None

Background: Exfoliative cytology for anal carcinoma screening in high risk patients is on the rise. However, no consensus guidelines have been established to direct clinical management based on the results of anal Papanicolaou (Pap) tests. Our study examined the rates of each Bethesda category on anal Pap tests, with particular focus on defining the characteristics of unsatisfactory cases, the prevalence of high risk (HR)-HPV types, and intraepithelial lesions on corresponding anal biopsies.

Design: The cytopathology database was searched for all anal cytology specimens and HPV DNA testing performed using Roche Cobas 4800 HR-HPV analysis. Concurrent or subsequent anal biopsies were then identified from the surgical pathology database.

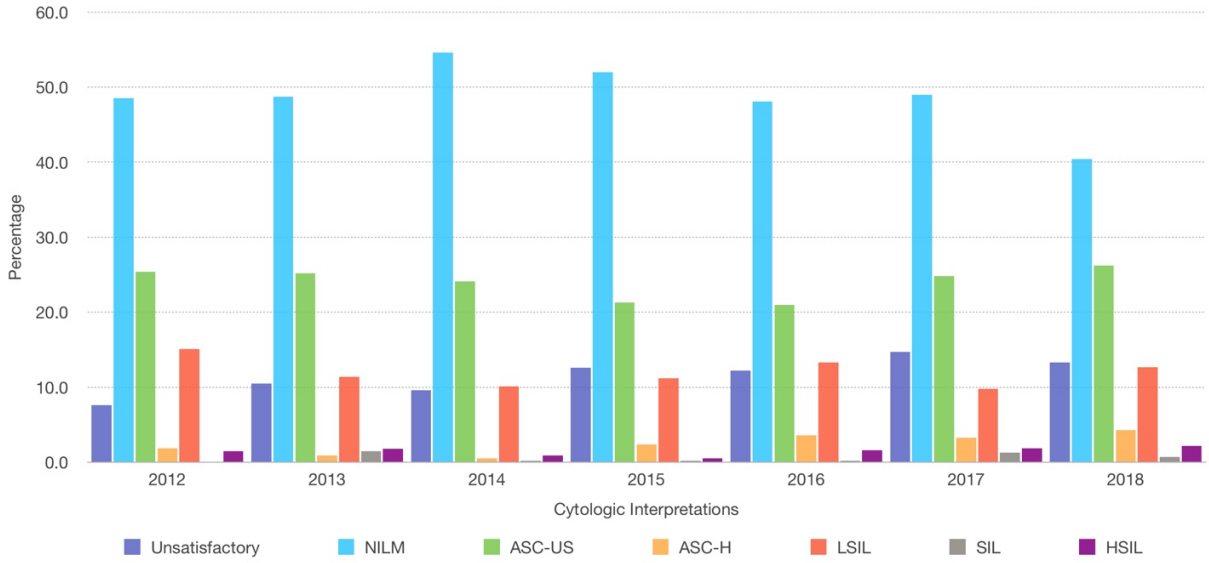
Results: 3710 total anal Pap tests performed from May 2012 to October 2018, 442 of which were deemed unsatisfactory (11.9%). The distribution of cytologic interpretations was relatively consistent year to year (Figure 1).

HPV co-testing began March 2015 and was performed in 2013 of 2340 (86%) cases. Indeterminate HR-HPV result (due to insufficient DNA to reach the threshold for a reliable negative result) occurred in 45 of 204 (22.1%) unsatisfactory cases and 15 of 1900 (0.8%) satisfactory cases. Of the remaining cases, HR-HPV positivity was seen in 52 of 159 (32.7%) unsatisfactory cases compared to 1075 of 1885 (57%) satisfactory cases.

In unsatisfactory cases, non-16/non-18 “other” HR-HPV types were most commonly identified: “other” only in 38 cases, “other” co-infected with HPV16/18 in 9 cases (5 with HPV16; 2 with HPV18; 2 with HPV16+18), 4 cases with HPV16 only, and 1 with HPV18 only. Of those patients with HR-HPV positivity on unsatisfactory anal cytology, 27 of 52 (51.9%) had anal biopsies within 6 months. No lesion was found in 14 (51.9%) biopsies. LSIL (AIN1) was diagnosed in 6 cases (22.2%), and HSIL (AIN2-3) was diagnosed in 7 cases (25.9%). The HR-HPV types seen in each anal biopsy diagnostic category is shown in Table 1.

Anal Tissue Biopsy Diagnosis	**HR-HPV Types**					
	16	18	Other	16+Other	18+Other	16+18+Other
No Biopsy	2 (3.8%)	1 (1.9%)	20 (38.5%)	2 (3.8%)	—	—
Benign anal mucosa	—	—	11 (21.2%)	1 (1.9%)	1 (1.9%)	1 (1.9%)
LSIL	1 (1.9%)	—	3 (5.8%)	2 (3.8%)	—	—
HSIL	1 (1.9%)	—	4 (7.7%)	—	1 (1.9%)	1 (1.9%)
Total	4 (7.7%)	1 (1.9%)	38 (73.1%)	5 (9.6%)	2 (3.8%)	2 (3.8%)

Figure 1 - 344



Conclusions: Non-16/Non-18 HR-HPV types are the most prevalent HPV type in unsatisfactory anal cytology specimens. 48.1% of patients with unsatisfactory anal cytology and positive HR-HPV were found to have LSIL/HSIL on anoscopic biopsy. This study suggests the importance of prompt follow up with repeat anal Pap testing for patients with unsatisfactory samples.

345 Thyroglobulin Measurements in Fine Needle Aspiration Specimens from Cervical Lymph Nodes for the Detection of Metastatic Thyroid Cancer: Institutional Analysis of Performance Characteristics

Caitlin Darrell[1], Michiya Nishino[2]
[1]University of Chicago Medical Center, Chicago, IL, [2]Beth Israel Deaconess Medical Center, Harvard Medical School, Boston, MA

Disclosures: Caitlin Darrell: None; Michiya Nishino: None

Background: In patients with a history of thyroid cancer, ultrasound (US) and fine needle aspiration (FNA) cytology are routinely utilized to evaluate cervical lymph nodes (LN) for metastatic thyroid cancer. Thyroglobulin (Tg) is produced by differentiated thyroid carcinoma and can be measured in FNA specimens as an alternative method of diagnosis, because Tg levels are expected to be undetectable in benign LN aspirates. Applying this attribute, we examined the performance characteristics of LN FNA Tg levels for the detection of metastatic thyroid cancer.

Design: The laboratory database was queried to identify patients with Tg measurements (Siemens Immulite) performed on cervical LN FNA specimens from 1/1/2016-12/31/2018. Tg levels >1 ng/mL were considered positive. Tg levels were correlated with concurrent FNA cytology and subsequent US or histologic follow-up, when available. Overall outcomes were classified as malignant (concurrent suspicious/malignant cytology; malignant follow-up histology) or benign (concurrent negative/nondiagnostic/atypical cytology with stable US follow-up; benign histology), respectively. Cases without follow-up were excluded from this analysis.

Results: 79 Tg assays on LN FNA samples with pathologic and/or sonographic follow-up were identified (Table), 44 (56%) of which had malignant outcomes. At Tg levels >1 ng/mL, the test achieved 84% sensitivity, 80% specificity, 84% positive predictive value, and 80% negative predictive value. Seven false negative cases (Tg negative; malignant outcome) were identified: papillary carcinoma (5), squamous cell carcinoma (1), and medullary carcinoma (1). Seven false positive cases (Tg positive; benign outcome) were detected: 5 were reported to be from level VI nodes (Tg range 2 – 9300 ng/mL), and 2 were from lateral neck nodes (Tg levels of 5 and 20 ng/mL, respectively).

Total number of lymph node FNA specimens classified by thyroglobulin measurement and outcome		
Tg measurement	Benign Outcome	Malignant Outcome
0-1 ng/mL	28	7
>1 ng/mL	7	37

Conclusions: FNA Tg measurements have high sensitivity and specificity for malignancy in the evaluation of lymph nodes that are clinically suspicious for thyroid cancer. Correlation with FNA cytology may help reduce the false negative rate (particularly for cancers that

do not express Tg, such as squamous cell carcinoma and medullary carcinoma) and false positive rate (e.g., by distinguishing residual central neck thyroid bed tissue from level VI lymph node sampling).

346 Evaluation of TERT Promoter and BRAF (V600E) Mutation Analysis on LBC-processed Thyroid Malignant Lesions

Marco Dell'Aquila[1], Maurizio Martini[1], Vincenzo Fiorentino[1], Teresa Musarra[2], Qianqian Zhang[1], Chiara Brunelli[1], Guido Fadda[3], Luigi Maria Larocca[4], Esther Rossi[5]
[1]Catholic University of Sacred Heart, Rome, Italy, [2]Roma, Italy, [3]University Cattolica Sacro Cuore, Rome, Italy, [4]Fondazione Policlinico Universitario A. Gemelli, Rome, Italy, [5]Catholic University, Rome, Italy

Disclosures: Marco Dell'Aquila: None; Maurizio Martini: None; Vincenzo Fiorentino: None; Teresa Musarra: None; Qianqian Zhang: None; Chiara Brunelli: None; Guido Fadda: None; Luigi Maria Larocca: None; Esther Rossi: None

Background: Papillary thyroid cancer (PTC) is the most common endocrine malignancy with an excellent prognosis in the majority of cases. About 5%-10% of patients have a particularly aggressive disease course with unknown explanation for the underlying genetic background. The adoption of molecular-based approaches to risk stratification for thyroid cancer hold significant promises. Several publications confirmed that *BRAF^{V600E}* and *TERT* promoter mutations can coexist in PTC and they can be involved in aggressiveness (recurrences and mortality). According to some studies, the stratification of patients using *BRAF^{V600E}* in combination with other molecular markers might be useful especially on thyroid fine needle aspiration cytology (FNAC) as an additional tool for malignant diagnoses and aggressive management. We analysed the role of *BRAF^{V600E}* and *TERT* mutations on thyroid FNAC lesions

Design: From January 2016 to August 2019, 219thyroid lesions, diagnosed by FNAC as indeterminate lesions(IL), suspicious for malignancy(SFM) and malignant(M), were enrolled. We also included 28 benign lesions (BL) as negative control. We performed molecular testing on both liquid based cytology and corresponding histology samples.

Results: The series included: benign-(28-12.8%), atypia/follicular lesion of undetermined significance (AUS/FLUS)-(78-35.6%), follicular neoplasm/suspicious for follicular neoplasm (SFN/FN)- (38-17.4%), suspicious for malignancy (SM)-(49-22.4%) and malignant (M)-(26-11.9%) cases. The series included 35*BRAF^{V600E}* mutated cases and 3cases with also *TERT* promoter mutations. Histologically, 49(22.6%) cases were benign, 165(76%) malignant, 2(0.9%)NIFTP. NIFTPs were originally diagnosed as FNs on FNAC. The majority of *BRAF^{V600E}* were in the SM and M(30 cases) showing a significant value ($p< 0.0001$); *TERT* mutated cases were 2M and 1SM. The *BRAF^{V600E}* mutated cases are mostly aggressive PTC variants (all TCV and hobnail PTC variants) with recurrences and lymph nodal metastases. Furthermore, the association between TERT and *BRAF^{V600E}* mutation is linked to distant metastases and poorly differentiated areas

Conclusions: TERT promoter mutations might be performed also on thyroid cytology and can be correlated with *BRAF^{V600E}*. Although *TERT* promoter mutations are extremely rare (1.3%),we found an association with 1)*BRAF*, 2) aggressiveness and 3) poorly differentiated component. A mortality risk stratification of PTC based on the *BRAF^{V600E}* and TERT genotype combination can be foreseen also on thyroid FNAC.

347 The Evaluation of Vaginal Pap Following Hysterectomy for Benign and Malignant Diseases: A Test Performance Pilot Study

Alice Dobi[1], Ming Zhang[2], Eugene Smith[2]
[1]Pennsylvania Hospital, Philadelphia, PA, [2]Penn Medicine, Philadelphia, PA

Disclosures: Alice Dobi: None; Ming Zhang: None; Eugene Smith: None

Background: Vaginal Pap/cytology tests following hysterectomy, constitutes an important screening and surveillance method to assess for vaginal precancerous lesions or tumor local recurrence. Some studies have shown little diagnostic efficacy and lack of cost effectiveness of these tests. However, there are limited data regarding the test performance of vaginal Pap, particularly using 2014 Bethesda system terminology, stratified for different underlying hysterectomy etiologies.

Design: The archived data (between 01/01/2015-07/01/2018) was analyzed based on primary hysterectomy diagnoses: benign (e.g. leiomyoma etc.) and malignant diseases, which was sub-classified into cervical squamous cell malignancy, cervical glandular cell malignancy, endometrial malignancy and vaginal malignancy. Cytologic diagnoses were made using 2014 Bethesda system terminology. Concurrent high-risk HPV testing and follow-up biopsy results were correlated.

Results: 178 patients (22 with benign and 156 with malignancy hysterectomy) contributed totally 548 Pap tests in our study. Rate of unsatisfactory specimen was markedly higher in cases with history of endometrial malignancy (16.9%) compared with cervical squamous malignancy (5.3%), cervical glandular malignancy (5.2%) and vaginal malignancy (5.6%). At least one abnormal cytology was identified in 5.7% of cases for benign hysterectomy, 51.5% for cervical squamous malignancy; 50% for vaginal, 16% for cervical glandular and 16.9%

for endometrial malignancy. ASCUS is the most common abnormal cytology in all groups, followed by LSIL in cervical squamous malignancy, AGC in cervical glandular and endometrial malignancy. HSIL was detected in 9 cases (4.3%) in cervical squamous malignancy, 60% of follow-up biopsy showing VAIN 3 or carcinoma. AGC or AIS/adenocarcinoma was found in 19 cases (9.7%) of endometrial malignancy groups, all biopsies are positive for recurrent adenocarcinoma. High-risk HPV was detected in 38% of cases with a history of primary vaginal malignancy, followed by 28.8% of cervical squamous malignancy, while lower rate (around 15%) in other groups.

Conclusions: The prevalence rate of abnormal vaginal Pap was significantly higher in women with a prior malignant hysterectomy, especially for cervical squamous lesions. The abnormal vaginal glandular cytology correlate with endometrial tumor local recurrence. Our pilot study reinforces the view that vaginal cytologic screening is beneficial for women who have had a hysterectomy for malignant diseases.

348 Practical Diagnostic Utility of Thyroid Fine Needle Aspiration Cell Blocks

Jacob Edens[1], Momal Chand[1], Shelby Miller[2], Monika Bhatt[3], Ian Anderson[1], Ishaq Azeem Asghar[3]
[1]St John Hospital and Medical Center, Detroit, MI, [2]Ascension St. John Hospital, Grosse Pointe Woods, MI, [3]Ascension St. John Hospital, Detroit, MI

Disclosures: Jacob Edens: None; Momal Chand: None; Shelby Miller: None; Monika Bhatt: None; Ian Anderson: None; Ishaq Azeem Asghar: None

Background: Thyroid fine needle aspiration is a powerful screening tool for assessing solitary thyroid nodules. Generally, morphologic evaluation of smears yields an accurate diagnosis; however, there are cases when it is beneficial to have a cell block so that ancillary studies like immunohistochemistry may be performed. Cytologic diagnoses guide clinical decisions, so it is important that accurate and efficient diagnoses be rendered. This study evaluates the diagnostic utility of the cell block in the evaluation of thyroid FNAs.

Design: A retrospective chart review of every thyroid FNA specimen from 1/2014 to 7/2019 was performed, and the following data were recorded: turn-around time (in days), diagnosis using the Bethesda criteria, if a cell block was prepared and whether it was contributory to the diagnosis or not. Median with range was calculated as the measure of central tendency and data were analyzed using chi-squared analysis and the Mann-Whitney U-test.

Results: Of the 2321 specimens, 40.2% (933) had cell blocks of which only 0.3% (7) were contributory to the diagnosis and immunohistochemistry was used for only 2 cases. For cases with cell blocks, the median turnaround time was 1 day with a range of 0 to 18 days and the median turnaround time without cell blocks was 0 with a range of 0 to 9 days. It was determined that cases with cell blocks were significantly more likely to have a turnaround time >1 day (65%, p<0.01) or >3days (25.4%, p<0.01).

	Median Turn-Around Times			
	Non-Diagnostic	Benign	Malignant	Indeterminant
Specimens with cell block	1	1	1	2
Range	[0,6]	[0,12]	[0,18]	[0,8]
Specimens with no cell block	0	0	0	0
Range	[0,7]	[0,6]	[0,9]	[0,3]
	Probability of Turnaround Times Beyond Chance			
	Same day	1 day	2 days	3+ days
Specimens with cell block	1.10%	65.00%	8.50%	25.40%
p-value				<0.01
Specimens with no cell block	66.40%	12.10%	11.60%	10.00%
p-value				<0.01

Figure 1 - 348

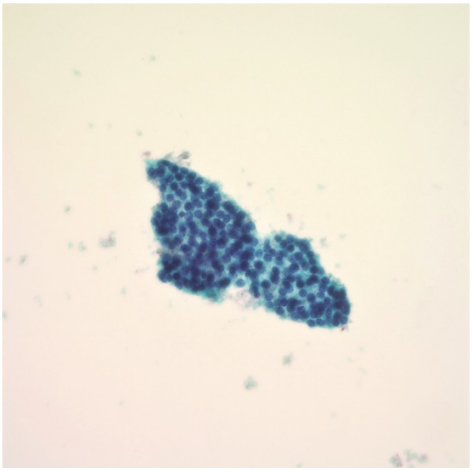
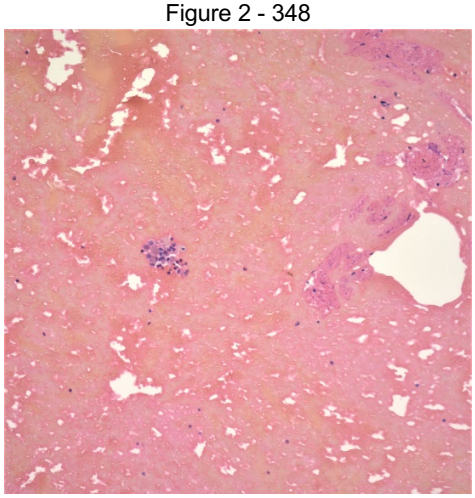


Figure 2 - 348



Conclusions: The diagnostic utility of cell block for thyroid FNAs was found to be very low. Of the 7 cases that were considered to be contributory to the diagnosis, ancillary tests were performed on only 3. The addition of a cell block added at least 1 day to the turnaround time in all diagnostic strata. The additional time causes patients to await the diagnosis of this screening test even for non-diagnostic studies. The increased turnaround time and resource and manpower expenditure could perhaps be avoided if cell blocks were produced as needed if the results of the smear were ambiguous or if ancillary tests were needed to confirm the diagnosis.

349 The Afirma Gene Sequencing Classifier in Cytologically Indeterminate Thyroid Nodules - Experience of a Single Institution

Arianne Foster[1], Michael Lai[1], William Klump[2], Lisa Reid[2], Shuyue Ren[2]
[1]Cooper Medical School of Rowan University, Camden, NJ, [2]Cooper University Hospital, Camden, NJ

Disclosures: Arianne Foster: None; Michael Lai: None; William Klump: None; Lisa Reid: None; Shuyue Ren: None

Background: The Afirma Gene Expression Classifier (GEC) was developed to provide an assessment of benignity or malignancy in thyroid nodules by fine needle aspiration; however, the test was found to be sensitive, but not specific. Studies have reported that the newer Afirma Gene Sequencing Classifier (GSC), RNA next-generation sequencing, has improved specificity, while preserving positive predictive value (PPV) and sensitivity. The purpose of this study is to assess the Afirma GSC performance at a single academic tertiary medical center.

Design: Retrospective analysis was performed on thyroid nodules diagnosed by Bethesda System for reporting thyroid cytopathology as category III (Atypia of undetermined significance [AUS]/Follicular Lesion of Undetermined Significance [FLUS]) and category IV (suspicious for Follicular Neoplasm [SFN]/Follicular Neoplasm [FN]) evaluated with Afirma GSC between June 2018 and June 2019 at a Radiology practice in a single academic medical center. Positive predictive value (PPV), negative predictive value (NPV), specificity, sensitivity, false positive and false negative calculations were performed. Statistical analysis was also performed on Bethesda III and IV categories individually.

Results: This study evaluated 114 nodules (AUS/FLUS and SFN/FN) from 105 patients. The GSC classified 42/114 (36.8%) nodules as suspicious, and malignancy was found in 16/23 (69.6%) of the suspicious nodules that continued to surgical resection. The GSC had 69.6% PPV (16/23), 91.0% specificity (71/78), 98.6% NPV (71/72) and 94.1% sensitivity (16/17). The high PPV of the GSC was sustained in both Bethesda category III (66.7%, 12/18) and Bethesda category IV (80.0%, 4/5) nodules. These results are similar to previous other studies (Endo et al, 2019. and Harrell et al, 2019). Compared to ThyroSeq NGS, another molecular test for indeterminate thyroid nodules performed at our institution, the PPV and specificity for GSC was 69.6% and 91.0% compared to the overall PPV and specificity of 70% and 85% for ThyroSeq NGS.

Table1. Results of GSC performed on thyroid nodules

	Sensitivity	*Specificity*	*NPV*	*PPV*	*n*	*TP*	*FP*	*TN*	*FN*
All Nodules	94.12%	91.03%	98.61%	69.57%	95	16	7	71	1
Bethesda III Nodules	92.31%	91.67%	98.51%	66.67%	85	12	6	66	1
Bethesda IV Nodules	100.00%	85.71%	100.00%	80.00%	11	4	1	5	0

Conclusions: Afirma GSC results from this Radiology practice in a single tertiary academic medical center show that the NPV and sensitivity are maintained, while PPV and specificity are improved compared to previously reported GEC results. Implementation of the GSC as standard protocol may significantly reduce surgical interventions and improve patient care.

350 Validation of PD-L1 Performance in Cytological Samples of Non-Small Cell Lung Carcinoma Obtained from Early-Stage Resection Specimens

Gerard Frigola[1], Naiara Vega[1], Anna González-Carreras[1], Cristina Teixido[1], Jose Ramirez[1], Daniel Martinez[2]
[1]Hospital Clinic, Barcelona, Spain, [2]Barcelona, Spain

Disclosures: Gerard Frigola: None; Naiara Vega: None; Anna González-Carreras: None; Cristina Teixido: None; Jose Ramirez: None; Daniel Martinez: None

Background: PD-L1 is a biomarker that identifies patients eligible immunotherapy. In non-small cell lung cancer (NSCLC) it is accepted that tumors regardless PD-L1 status are eligible to combination of pembrolizumab and chemotherapy as first line treatment although patients with at least TPS 50% could benefit with monotherapy alone. In order to stratify which patients could benefit with monotherapy and because NSCLC diagnosis is often made only on cytological samples (30%), establishing a cytology-histology correlation on the same samples is essential to determine the technical feasibility of testing PD-L1 in cytology.

Design: The aim of the study was to validate PD-L1 testing in cytological samples on a selected cohort of early-stage NSCLC.

Fine needle aspiration (FNA) was performed directly on NSCLC resection specimens, obtaining representative smears and CB with neoplastic cells from each case. A Papanicolaou or Diff-Quik-stained smear, a CB section and a representative histological section from the tumor were stained with a PD-L1 antibody (clone 22C3, Dako pharmDx). Tumor proportion score (TPS) was assessed by two pathologists. In discrepant determinations, consensus was reached. Samples with <50 neoplastic cells were excluded. Average TPS values were compared with the Kruskal-Wallis test, and correlations between smears, CB and histology were assessed with Cohen's κ coefficient.

Results: We evaluated 108 samples from 36 early-stage NSCLC patients. Average age was 68 years (range, 42-82), 28 patients were males, and 5 were never-smokers. A TPS ≥50% was observed in 13/36 cases (36,1%); 11/36 cases (30,6%) showed TPS 1-49%; and 12/36 cases (33,3%) had a TPS <1%. No significant differences were seen in the average TPS among smears, CB and histology groups (p=0,231), but smears tended to have lower TPS values. No false positive smears were seen. The only false positive CB had a TPS value of 1%. False negative rates were 20% for smears (3/15) and 26.67% for CB (4/15). In 3 of the 4 false negative CB, the difference of TPS between histology and CB was below 4% on average, and 2 of them only displayed 50-100 evaluable neoplastic cells. PD-L1 status correlation was substantial between smears and histology (κ=0,708) and between CB and histology (κ=0,733).

		HISTOLOGY TPS (%)			AGREEMENT	KAPPA
		<1	**1-49**	**≥50**		
SMEAR TPS (%)	**<1**	12	1	2	29/36 (80,56%)	0,708*
	1-49	0	10	4		
	≥50	0	0	7		
CELL BLOCK TPS (%)	**<1**	11	4	0	28/34 (82,35%)	0,733*
	1-49	1	6	1		
	≥50	0	0	11		
* Substantial correlation						

Conclusions: We have observed a good correlation between histology and cytology samples regarding PD-L1 assessment. With these results, we believe that FNA direct smears from advanced stage of NSCLC could be used for PD-L1 assessment.

351 The Emerging Role of Ancillary Studies in the Milan System for Reporting Salivary Gland Cytopathology

Matthew Gabrielson[1], Ryan Lu[1], Zahra Maleki[2]
[1]Department of Pathology, The Johns Hopkins Medical Institutions, Baltimore, MD, [2]Johns Hopkins Hospital, Baltimore, MD

Disclosures: Matthew Gabrielson: None; Ryan Lu: None; Zahra Maleki: None

Background: Cell blocks prepared from fine-needle aspirates (FNAs) are commonly used for additional ancillary studies, such as immunocytochemical stains, fluorescence in situ hybridization, and molecular testing in order to increase diagnostic yields. FNAs of salivary gland lesions are relatively uncommon and rendering a definitive diagnosis can be challenging due to heterogeneity of the lesions. Cell blocks are rarely prepared in salivary gland cytopathology, and there are limited experiences on utilizing them in this setting. In this study, we evaluate the role of ancillary studies in diagnosis of salivary gland lesions and its impact on reporting the cases applying the Milan System for Reporting Salivary Gland Cytopathology (MSRSGC).

Design: This retrospective study evaluated whether ancillary studies performed on cell blocks from FNAs were reliable in guiding diagnosis and patient management for salivary gland lesions. We analyzed data for all available salivary gland lesions in which an FNA cell block was ordered and utilized for ancillary studies over the past 20 years at a large tertiary care hospital and academic center. Clinical information was gathered for each case, including the diagnosis by cytology, MSRSGC category, ancillary studies performed on the cell block, and subsequent surgical follow-up and surgical pathology diagnosis.

Results: Sixty four (64) cases that met these criteria were identified, including cases from 37 male patients and 27 female patients, ranging in age from 2 to 92 years old. In 24 of 64 cases, immunohistochemistry performed on cell block yielded a malignant diagnosis, and prompted subsequent surgical follow-up. In all cases where surgical follow-up was indicated and performed, the malignant diagnosis established with immunohistochemistry performed on cell block was confirmed with surgical diagnosis. In 40 of 64 cases, immunohistochemistry performed on cell block supported a benign diagnosis and prevented unnecessary surgical intervention.

Milan Category	Number of Cases	Cytology Diagnosis	Ancillary Studies on Cell Block	Surgical Diagnosis
I	(n=3)	Hypocellular cyst contents composed of histiocytes, lymphocytes, neutrophils	Positive for CD68; Negative for AE1/AE3	Cystic low grade mucoepidermoid carcinoma
		Non-diagnostic specimen		No surgical follow-up
		Predominantly histiocytes consistent with cyst contents	Positive for HAM56	No surgical follow-up
II	(n=1)	Caseating granuloma	Positive acid-fast stain	No surgical follow-up
III	(n=2)	Atypical mononuclear cells in background of lymphocytes	Negative for CD15 and CD30; Equivocal for CD68 and HAM56	No surgical follow-up
		Scant specimen with rare atypical epithelial cells in background of lymphocytes	Negative for mucicarmine	No surgical follow-up
IVa	(n=1)	Schwannoma	Positive for S100	Schwannoma
IVb	(n=3)	Basaloid neoplasm, favor salivary gland origin. No definitive lymphoid tissue present, SUMP	Negative for thyroglobulin	No surgical follow-up
		Low grade salivary gland neoplasm, favor pleomorphic adenoma, SUMP	Neoplastic epithelial cells positive for AE1/3 and C-KIT; Neoplastic myoepithelial cells positive for p63, AE1/3, S100, and calponin; Negative for synaptophysin	Poorly differentiated neuroendocrine neoplasm most consistent with recurrent esthesioneuroblastoma
		Cellular epithelial neoplasm of salivary origin, SUMP	Negative for mucicarmine	Invasive carcinoma with neuroendocrine differentiation
V	(n=0)	No cases fell within this category		
VI	(n=17)	Adenocarcinoma	Positive for CK7; negative for CK20	Salivary duct carcinoma
		Metastatic malignant melanoma	Positive for HMB 45	Metastatic melanoma
		Poorly differentiated non-small cell carcinoma	Negative for thyroglobulin	Acinic cell carcinoma
		Mucoepidermoid carcinoma, low grade	Positive mucicarmine stain	No surgical follow-up
		Malignant neoplasm	Positive for CD56, chromogranin, synaptophysin (weakly and focally), and CD56. negative for AE1/AE3, CD45, S100, p63, L-Actin, actin, MGN, and CD45	Metastatic malignant oligodendroglioma
		Extranodal marginal zone lymphoma of mucosa-associated lymphoid tissue	Positive for CD20; Negative for CD10, BCL-6, CD5, CD23, and cyclin D1	No surgical follow-up
		High-grade carcinoma	Positive for cytokeratin; Negative for chromogranin, calcitonin, s-100, HMB-45, thyroglobulin, mucin	High grade adenocarcinoma
		Small cell carcinoma	Positive for CD56, chromogranin, and synaptophysin; negative for O13, CK20, CD3, CD10, CD20, CD45, Kappa and Lambda light chains; equivocal for AE1/AE3	No surgical follow-up
		Salivary gland neoplasm, consistent with well-differentiated acinic cell carcinoma	Positive for pan cytokeratin; Negative for GFAP, S100, smooth muscle actin, and mucicarmine	No surgical follow-up
		Squamous cell carcinoma with keratinization	Positive for P16 and HPV	HPV-related squamous cell carcinoma

		Involvement with patient's known myeloma	Positive for CD138 and kappa; negative for lambda	No surgical follow-up
		Non-small cell carcinoma morphologically consistent with metastasis from patient's known papillary thyroid carcinoma	Positive for thyroglobulin	No surgical follow-up
		Poorly differentiated adenocarcinoma	Positive for cytokeratin, AE1/AE3, CAM5.2, and mucicarmine; negative for S100, HMB45, and Melan A	Invasive salivary duct carcinoma
		Metastatic squamous cell carcinoma, consistent with patient's known primary	Positive for P63 and CAM5.2	Poorly differentiated squamous cell carcinoma
		Squamous cell carcinoma	Negative for P16 and HR HPV	No surgical follow-up
		Large B cell lymphoma	CD20 stains confluent sheets of large B; dimly positive for BCL-2, and lack CD5 and CD10. Ki-67 staining demonstrates an elevated proliferative index of 50-60%. CD23, NKX3.1 are negative.	No surgical follow-up

Conclusions: Our retrospective analysis demonstrates that ancillary studies performed on FNA cell blocks of salivary gland lesions can reliably help to characterize these lesions and guide appropriate clinical management.

352 Secretory Carcinoma of the Salivary Gland, a Rare Entity: An International Multi-Institutional Study

Matthew Gabrielson[1], William Faquin[2], Zubair Baloch[3], Vickie Jo[4], Fabiano Callegari[5], Ivana Kholovà[6], Sharon Song[3], Satu Tommola[7], Guido Fadda[8], He Wang[9], Syed Ali[10], Esther Rossi[11], Liron Pantanowitz[12], Zahra Maleki[13]
[1]Department of Pathology, The Johns Hopkins Medical Institutions, Baltimore, MD, [2]Massachusetts General Hospital, Harvard Medical School, Boston, MA, [3]Hospital of the University of Pennsylvania, Philadelphia, PA, [4]Brigham and Women's Hospital, Harvard Medical School, Boston, MA, [5]UNIFESP, São Paulo, SP, Brazil, [6]University of Tampere, Tampere, Pirkanmaa, Finland, [7]Department of Pathology, Fimlab Laboratories, Tampere, Tampere, Finland, [8]University Cattolica Sacro Cuore, Rome, Italy, [9]Rutgers - Robert Wood Johnson Medical School, New Brunswick, NJ, [10]Johns Hopkins Medical Institutions, Baltimore, MD, [11]Catholic University, Rome, Italy, [12]University of Pittsburgh, Wexford, PA, [13]Johns Hopkins Hospital, Baltimore, MD

Disclosures: Matthew Gabrielson: None; William Faquin: None; Zubair Baloch: None; Ivana Kholovà: None; Sharon Song: None; Satu Tommola: None; Esther Rossi: None; Liron Pantanowitz: None; Zahra Maleki: None

Background: Secretory carcinoma (SCA) of the salivary gland is a rare tumor first characterized in 2010 as an entity distinct from acinic cell carcinoma. The published literature on cytomorphologic features of SCA on fine needle aspiration (FNA)is limited to mostly case reports or small series.

Design: After each institutional approval, the electronic data of eight large academic institutions (five in the United States, two in Europe (Italy and Finland) and one in Brazil) were retrospectively searched for FNA cases of SCA that were confirmed by histology. Patients' demographics, imaging findings, molecular alterations, cytomorphologic characteristics and immunostaining profile were recorded. For FNA interpretation, The Milan System for Reporting Salivary Gland Cytopathology was applied.

Results: Thirty two cases from eight institutions were identified including 12 males and 20 females ranging in age from 13 to 80 (mean=49.25 years). The tumors presented as solid or occasionally cystic masses, which were hypoechoic on imaging **(Table 1).** Although parotid remained the most common primary site of the tumor, lesions arising in the minor salivary glands of oral cavity and trachea were also seen. The tracheal tumor that arose from minor salivary gland invaded the thyroid and cervical lymph nodes at the time of initial presentation. Overall, the aspirates were cellular comprising of mainly large tissue fragments with focal papillary architecture and occasionally acinar-like formations in a mucinous to vacuolated background. The cells were medium in size with abundant finely granular, eosinophilic or vacuolated cytoplasm, round to oval, centrally or eccentrically placed nuclei with a single small nucleolus. GATA-3, mammaglobin and S-100 protein were positive whenever performed. *ETV6-NTRK3*, t(12;15)(p13;q25), rearrangement was detected in 30 cases by Flouroscent in situ hybridization (n=29) and NGS (thyroseq)(n=1). Regional lymph node metastasis and distant metastasis to the brain, liver and mediastinum were noted in three patients. One patient died of the disease.

	Secretory Carcinoma on FNA	
Patients' Demographics	Males (n=12) Females (n=20) Age 13-80 (mean= 49.25)	
Clinical Presentation	Parotid, neck, intraoral or thyroid mass, firm, slow growing, tender or painless	
Imaging	Hypoechoic nodule, ovoid lesion with poorly defined borders Poly lobular, iso-hypoechoic nodule PET positive lymph nodes	
Anatomic site	Parotid (n=21) Submandibular (n=3) Right buccal minor salivary glands (n=2) Minor salivary gland of trachea invading thyroid with lymph node metastasis (n=1) Oral cavity (n=1) Neck mass (n=1)	
Cytomorphology	**Cellular aspirates** **Architecture:** Predominantly sheets, clusters, and crowded groups of neoplastic cells, micro and thin papillary pattern, transgressing vessels, acinar formation and single cells **Neoplastic cells:** Medium sized epithelial cells with minimal or no pleomorphism, ductal-type, polygonal, plasmacytoid or monotonous oncocytic-type cells **Nucleus:** Round to oval uniform nuclei with small but notable nucleoli, centrally or eccentrally placed, finely coarse and hyperchromatic chromatin, smooth nuclear contour, rare binucleation, rare nuclear inclusions, mitosis: rare to absent **Cytoplasm:** Moderate to abundant cytoplasm, finely granular, clear or eosinophilic particularly on cell blocks, intracytoplasmic vacuoles with variable size predominantly large and often single **Background:** variable from abundant mucin to clean with granular and amorphous debris, cystic, vacuoles with variation in quantity and size, scattered lymphocytes, multinucleated giant cells, hemosiderin-laden macrophages, metachromatic matrix in fibrillary and spherical shapes, occasionally hemorrhagic, no necrosis	
Immunohistochemistry	Positive	Mammaglobin, GATA-3, S100 protein, AE1/AE3, CK7, SOX10, CK8, CK5/6, BRST2, calponin, - (not immunostains)
	Negative	DOG-1, P63, PAX8, P40, TTF-1, Thyroglobulin, Androgen receptor, GCDFP-15,beta-catenin, CK20,ER, SMA
Molecular profile	*ETV6-NTRK3*, t(12;15)(p13;q25) (30/30) by Flouroscent in situ hybridization (n=29) and Next Generation Sequencing (thyroseq) (n=1).	
Milan System for Reporting Salivary Gland	Malignant (n=15) Suspicious for malignancy (10) Salivary gland neoplasm of uncertain malignant potential (n=5) Atypia of undetermined significance (n=2)	
Metastasis	Regional	Cervical lymph nodes (8/32), 3 with extra nodal extension, thyroid (1)
	Distant	Brain (n=1), liver (n=1), lung and mediastinum (n=1)
Death	1. 53 year old man, 13 months after the diagnosis	

Table 1. Cytomorphologic, imaging, and clinical findings of Secretory carcinoma on aspirated material from eight large academic institutions, a multi-institutional international collaboration.

Conclusions: Our study shows that SCA, a rare malignancy of middle aged patients, may arise from minor salivary glands in addition to parotid and submandibular gland. Although considered low grade, regional lymph node metastasis at the time of initial presentation is not uncommon. Familiarity with cytomorphologic features of SCA would enhance the diagnostic accuracy for better clinical management and outcome.

353 Adequacy Evaluation of Pancreatic Adenocarcinoma Specimen for Next-Generation Sequencing Test Acquired by Endoscopic Ultrasound-Guided Fine-Needle Aspiration and Fine-Needle Biopsy

Qiong Gan[1], Emmanuel Coronel[1], Manoop Bhutani[1], Brian Weston[1], Anirban Maitra[1], Jeffrey Lee[1], John Stewart[1], Sinchita Roy-Chowdhuri[1]
[1]*The University of Texas MD Anderson Cancer Center, Houston, TX*

Disclosures: Qiong Gan: None; John Stewart: None; Sinchita Roy-Chowdhuri: None

Background: Endoscopic ultrasound guided fine-needle aspiration (EUS-FNA) is the mainstay for tissue acquisition for the diagnosis of pancreatic ductal adenocarcinoma (PDAC); however the role of EUS-FNA in biomarker (BM) testing for personalized treatment or precise chemotherapy has not been well established. Recently, EUS-fine needle biopsy (EUS-FNB) using flexible biopsy needles is being used in PDAC for tissue acquisition in an effort to increase diagnostic yield for BM testing. Herein, we reviewed concurrently acquired EUS-FNA and EUS-FNB samples from PDAC to evaluate the diagnostic yield and adequacy for BM testing.

Design: PDAC cases diagnosed by specimens acquired through concurrent EUS-FNA/FNB techniques from 1/1/2018-5/15/2019 after institutional IRB approval were reviewed. Smears were prepared from EUS-FNA sampling and cell blocks (CB) were prepared from EUS-FNB sampling using standard procedures. Rapid on-site evaluation was performed in all cases. Tumor cells on smears and CB sections were reviewed and evaluated for adequacy for BM testing including next-generation sequencing (NGS) and immunohistochemical assays (IHC) by two cytopathologists independently. The adequacy criterion for NGS was: tumor fraction ≥20% and ≥ 5000 tumor cells; and the adequacy criterion for IHC was: ≥50 tumor cells on CB section.

Results: 1. There were 26 cases concurrently sampled by both EUS-FNA and EUS-FNB techniques during this study period. ProCore® and Acquire™ needles were used in 10 (38.4%) and 16 (61.5%) cases separately. Although the size was small, Acquire needles showed slight better performance than ProCore needle (details in Table 1).

2. Smears for all 26 (100%) FNA cases were diagnostic for PDAC, compared to only 20 (76.9%) FNB samples were diagnostic for PDAC.

3. By using FNB alone, 11 cases would be suitable for both NGS and IHC; while by combining FNA and FNB, 16 cases would be suitable.

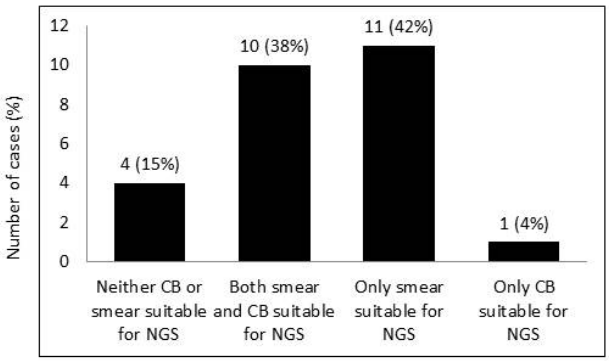
4. For NGS evaluation, 10 cases would be adequate using either smears or CB, 11 cases would be adequate only on smears, 1 case would be adequate on only CB , while 4 cases would be inadequate on either smears or CB (Figure 1)

Table 1. Summary of needle type, size, and passes

Needle type	ProCore	10
	Neither CB or smear suitable for NGS	2 (20%)
	Both smear and CB suitable for NGS	4 (40%)
	Only smear suitable for NGS	4 (40%)
	Acquire	16
	Neither CB or smear suitable for NGS	2 (12.5%)
	Both smear and CB suitable for NGS	6 (37.5%)
	Only smear suitable for NGS	7 (43.8%)
	Only CB suitable for NGS	1 (6.2%)
Needle size		
	22G	8
	25G	8
	22G and 25G	10
Pass		
mean (range)	3.84 (2 to 7)	

Figure 1 - 353

Figure 1. Evaluation of EUS-FNA and EUS-FNB cases for NGS adequacy



Abbreviations: CB, cell block; EUS, endoscopic ultrasound guided fine-needle aspiration or fine needle biopsy; NGS: next generation sequencing

Conclusions: Our results show smears sampled by EUS-FNA provide higher tumor fraction than CB sampled by EUS-FNB, which is better suited for NGS analysis, while the advantage of the latter specimen is more suitable for IHC-based BM testing. In summary, these two types of tissue acquisition techniques complement each other to provide adequate material for BM testing in PDAC.

354 Comparison of Afirma Gene Expression Classifier with Gene Sequencing Classifier in Indeterminate Thyroid Nodules: A Single-Institutional Experience

Yipeng Geng[1], Josephine Aguilar-Jakthong[2], Neda Moatamed[3]
[1]University of California Los Angeles, Los Angeles, CA, [2]University of California Los Angeles, Grass Valley, CA, [3]David Geffen School of Medicine at UCLA, Los Angeles, CA

Disclosures: Yipeng Geng: None; Josephine Aguilar-Jakthong: None; Neda Moatamed: None

Background: The Afirma test has been used in the diagnosis of cytologically indeterminate thyroid nodules to reduce diagnostic uncertainty and unnecessary surgeries. Gene Sequencing Classifier (GSC) was developed to improve the positive predictive value (PPV) and overall test performance of Gene Expression Classifier (GEC). Compared to GEC, there are currently limited data on the test performance of GSC. Here we present our institutional experience comparing the performance of first generation assay of Afirma (GEC) with the second generation assay (GSC).

Design: Retrospective analysis was performed on all Bethesda categories III and IV cytology thyroid nodules tested with GEC (from 08/2015 to 07/2017) and GSC (from 08/2017 to 02/2019). Test performances were evaluated by surgical pathology outcomes.

Results: A total of 168 cases were tested with GEC. 49% (82/168) were reported as benign. Fourteen of these cases had surgical follow-up with 11 benign, 1 noninvasive follicular thyroid neoplasm with papillary-like nuclear features (NIFTP) and 2 malignant diagnoses on histology. 51% (86/168) had suspicious GEC result. Fifty-seven of these suspicious GEC cases had surgical follow-up with 28 benign, 9 NIFTP and 20 malignant histology.

A total of 133 cases were tested with GSC. 61% (81/133) were reported as benign. Nine of these cases had surgical follow-up, all of which showed benign results. 32% (52/133) cases were tested as suspicious. Thirty-four of these cases with suspicious GSC had surgical follow-up with 14 benign, 5 NIFTP, and 15 malignant histology. The performances of the two tests were compared in Table 1. Based on molecular testing, surgical resection could have been prevented in 81 patients (61%) with GSC, compared to 79 patients (47%) with GEC test ($p < 0.05$).

Table 1. Test Performance of Afirma GEC and GSC in cytologically indeterminate thyroid nodules (PPV: positive predictive value, NPV: negative predictive value).

	GEC	GSC
Sensitivity	91%	100%
Specificity	28%	42%
PPV	51%	60%
NPV	79%	100%

Conclusions: Our experience with Afirma shows that the current version GSC has a better test performance. Also, our data is consistent with prior data that GSC identifies more cases as benign and therefore, reduces the number of unnecessary surgeries compared to the prior version, GEC.

355 Anaplastic Thyroid Carcinoma and the Value of Fine Needle Aspiration Cytology in Diagnosis and Molecular Analysis

Rachelle Gietzen[1], Cecilia Clement[2], Ranjana Nawgiri[2]
[1]University of Texas Medical Branch - UTMB, Galveston, TX, [2]University of Texas Medical Branch, Galveston, TX

Disclosures: Rachelle Gietzen: None; Cecilia Clement: None; Ranjana Nawgiri: None

Background: Anaplastic thyroid carcinoma (ATC), a highly aggressive form of thyroid cancer is seen in less than 2% of all thyroid cancers. Fine needle aspiration (FNA) plays an important role as a diagnostic modality of this rare cancer and in subsequent molecular testing. Identification of BRAF mutational analysis is essential for prognostic and theranostic purposes. It is crucial for pathologists to not just recognize the cytomorphology but also ensure the success of further molecular testing on these limited samples. We present a series of ATC cases with a focus on the cytomorphology and molecular testing done on cytology samples.

Design: We ran a search through the laboratory information system for a period between Jan 2017- July 2019. All cases that had a diagnosis of primary thyroid cancer on FNA were evaluated and those with a diagnosis of ATC were studied.

Results: Of the 903 cases of FNAs of the thyroid, 47 were primary thyroid cancer. Of these 36 were papillary carcinomas, 1 medullary carcinoma, and 6 ATC. Of the 6 ATCs, 3 had mutational analysis done on cytology specimens (2 on cytology smears and 1 on a cell block) of which two out of three were positive for the V600E mutation. 2 were deceased shortly after diagnosis and 1 was immediately lost to follow up (returned to home country).

Case 1	71 yo female	ATC with BRAF V600E was negative on smear
Case 2	68 yo male	ATC with BRAF V600E was positive on cell block
Case 3	48 yo male	ATC - lost to follow up (returned to home country)
Case 4	47 yo female	ATC - deceased
Case 5	52 yo male	ATC - deceased
Case 6	64 yo female	ATC with BRAF V600E was positive on smear

Figure 1 - 355

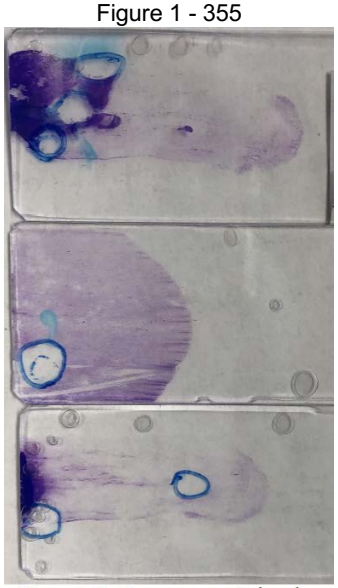


Figure 1. Tumor enriched areas micro dissected for molecular analysis

Figure 2 - 355

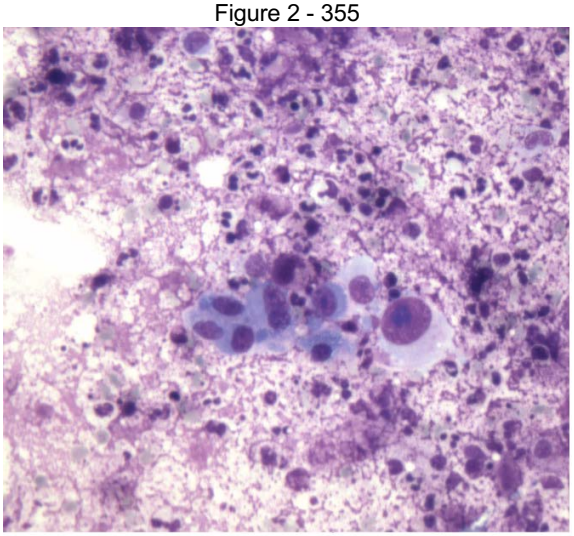


Figure 2. Romanowsky stained air dried smear showing malignant cells. (Anaplastic Thyroid Carcinoma)

Conclusions: The cytomorphological and clinical features are key in avoiding misclassification of this lethal cancer. A timely diagnosis with adequate sampling to ensure that the molecular testing can be successful is crucial. This review demonstrates cytology specimens are robust samples on which molecular testing can be done.

356 Molecular Alterations in Hurthle Cell Neoplasm: A Fine Needle Aspiration Study

Syed Gilani[1], Deepika Kumar[2], Rita Abi-Raad[1], Guoping Cai[3], Adebowale Adeniran[4]
[1]Yale University School of Medicine, New Haven, CT, [2]Yale New Haven Hospital, New Haven, CT, [3]Yale University, Wallingford, CT, [4]Yale University School of Medicine, Seymour, CT

Disclosures: Syed Gilani: None; Deepika Kumar: None; Rita Abi-Raad: None; Guoping Cai: None; Adebowale Adeniran: None

Background: Hurthle cell features are frequently seen on the fine needle aspiration (FNA) cytology and ranges generally from benign to malignant but often times poses a diagnostic challenge. Molecular alterations associated with these lesions are not well described. This study was conducted to evaluate and identify the molecular profile of Hurthle cell lesions classified as Hurthle cell neoplasm (HCN) on cytologic evaluation

Design: A total of 5655 FNAs were evaluated at our institution from January 2017 to June 30, 2019. Of those, 237 (4%) cases from 233 patients (F=188, M=45, Age (Mean $\pm$ S.D) =55.34 $\pm$ 16.0) were diagnosed as HCN. Molecular tests were done on various platforms depending on the preference of the referring endocrinologists.

Results: Molecular testing results were available in 62 (26.1%) cases by using different platforms: Thyroseq (n)=43 (positive=21), Afirma (n)= 5 (suspicious=4), ThyGenX and ThyraMIR (n)= 10 (positive=3) and Rosetta GX (n)=4 (suspicious=1). The most frequently encountered genetic alterations were gene fusion and multiple chromosome copy number alteration (n=9), followed by NRAS (n=4), KRAS (n=4), EIFIAX (n=2), and 1 each of GNAS, BRAF K601E, TERT, HRAS mutations were observed. One hundred and thirty-three cases (56.1%) had histologic follow up and of those 29 were classified as malignant, 37 as benign neoplasm (2 Noninvasive follicular thyroid neoplasm with papillary-like nuclear features: NIFTP and 35 adenomas) and 67 as negative. Nine out of twenty-nine malignant cases had undergone molecular testing (benign/negative=4 and suspicious/positive=5).

Molecular test		**Histologic resection**		
		Negative	Benign neoplasms	Malignant
THYROSEQ	Negative	4	0	4
	Positive	8 (TERT, EIFIAX, BRAFK601E, NRAS, GNAS, gene fusion and multiple chromosome copy number alteration x3)	4 (NRAS, gene fusion and multiple chromosome copy number alteration x3)	3 (KRAS, NRAS, gene fusion and multiple chromosome copy number alteration)
AFIRMA	Benign	0	1	0
	Suspicious	1	1	2
ThyGenX and ThyraMIR	Negative/benign	1	1	0
	Positive	2 (KRAS, MIR positive)	1 (HRAS)	0
Rosetta GX	Benign	0	0	0
	Suspicious	1	0	0

Conclusions: In this study we observed that Hurthle cell neoplasm is a heterogeneous group of lesions. Molecular alterations are relatively variable in HCN without any specific associations. These findings suggest a limited role of molecular results in indeterminate Hurthle cell lesions.

357 Root Cause Analysis of Indeterminate Diagnoses in Serous Fluids Cytopathology

Hamza Gokozan[1], Aparna Harbhajanka[2], Sandra Lyden[1], Claire Michael[3]
[1]University Hospitals Cleveland Medical Center, Case Western Reserve University, Cleveland, OH, [2]Cleveland, OH, [3]University Hospitals Cleveland Medical Center, Cleveland, OH

Disclosures: Hamza Gokozan: None; Aparna Harbhajanka: None; Sandra Lyden: None; Claire Michael: None

Background: A definitive diagnosis on serous fluids dictates further management of the patients. Recently the International System of Serous Fluids Cytopathology (ISSFC) proposed 5 categories: Nondiagnostic, Benign, Atypical, Suspicious and Malignant. The atypical/suspicious categories are problematic from the clinical perspective and trigger further diagnostic procedures adding both cost and morbidity to the patient. The goal of this study is to understand the root causes that contribute to these indeterminate diagnoses (ID).

Design: We searched our archives for serous fluids received by our cytopathology laboratory between 1/1/2017 and 6/30/2019. A single ThinPrep and cell block (CB) were prepared for each case. Root cause analysis of factors contributing to an ID was performed.

Results: A total of 911 fluids were received by the laboratory and diagnosed as: 9 Nondiagnostic (1%), 667 Negative (73.2%), 51 Atypical (5.6%), 27 Suspicious (3%) and 157 malignant (17.2%). More than one factor contributed to 38/78 ID. Low specimen volume defined as <50 cc was identified in 31 cases (23/51 atypical and 8/27 suspicious). Low cellularity was identified in 51 cases. IHC was performed on 11/51 and deemed noncontributory, whereas in 7/51 IHC highlighted the atypical cells but was not enough for a malignant diagnosis. Three cases (1 atypical, 2 suspicious) were simply deferred to concurrent biopsy. Eleven cases were called atypical, favor reactive mesothelium despite confirmatory IHC. A history of malignancy was noted in 35 cases. Atypical lymphocytes were reported in 4 cases and additional 3 were reported as suspicious despite concurrent flow cytometry. Four cases were worked up for the wrong differential diagnoses. CBs were low in cellularity despite adequate volume in 6 cases. Two mesotheliomas were under called.

Table 1: Root Causes Identified for the Cohort *: More than one factor contributed to 38/78 Indeterminate Diagnosis.

Root Causes by Category*	Atypical (n:51)	Suspicious (n:27)	Total (n:78)
Low cellularity only	26/51 (51%)	7/27 (25.9%)	33/78 (42.3%)
Low cellularity with confirmatory IHC	1/51 (1.9%)	6/27 (22.2%)	7/78 (9%)
Mesothelial cells with previous history of cancer	13/51 (25.5%)	0/27 (0%)	13/78 (16.7%)
Atypia favor reactive	6/51 (11.8%)	0/27 (0%)	6/78 (7.7%)
Volume less than 50 ml	23/51 (45%)	8/27 (29.6%)	31/78 (39.7%)
Low cellularity with nonconfirmatory IHC	2/51 (3.9%)	9/27 (33.3%)	11/78 (14.1%)
No ancillary testing done, defer to correlating biopsy	1 /51 (1.9%)	2/27 (7.4%)	3/78 (3.8%)
Lymphoid	4/51 (7.8%)	3/27 (11.1%)	7/78 (9%)
Case worked up for wrong differential diagnosis not timely correlated with the concurrent biopsy	1/51 (1.9%)	3/27 (11.1%)	4/78 (5.1%)

Conclusions: Identified root cases are the following: 1) Low cellularity was the most important contributing factor. 2) Low volume contributed to 31% of these cases and was a result of multiple factors including: Confusion over the appropriate collection containers that can be transferred to lab and clinicians being unaware of the necessity of an adequate volume. 3) Improper cell block processing as an additional cause of low cellularity and some cases may have benefited from a second CB. 4) Some pathologists tend to report reactive mesothelium as atypical when they perform confirmatory IHC or in the setting of malignant history and 5) there is a tendency to under call malignant mesothelioma.

358 Workup of Adenocarcinoma in Serous Fluids: Comparative Performance Characteristics of MOC31, B72.3 and mCEA as First-Line Markers

Hamza Gokozan[1], Claire Michael[2], Aparna Harbhajanka[3]
[1]University Hospitals Cleveland Medical Center, Case Western Reserve University, Cleveland, OH, [2]University Hospitals Cleveland Medical Center, Cleveland, OH, [3]Cleveland, OH

Disclosures: Hamza Gokozan: None; Claire Michael: None; Aparna Harbhajanka: None

Background: Serous fluids are frequently the first manifestation of a malignancy and a definitive diagnosis would spare the patient additional morbid procedure. General epithelial markers such as MOC31, B72.3 and mCEA are commonly used for initial workup of suspected adenocarcinomas (ADC), and are further confirmed by lineage specific markers. However, not all ADCs have different preferential reactions to these markers. This study aims to compare the performance characteristics of three most commonly used IHC to identify the best possible combination for investigating ADCs of different suspected site.

Design: We conducted a retrospective analysis of serous fluid cytology cases encountered between January 2017 and May 2019. Diagnosis, site and IHC information were collected and major performance characteristics including sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) were analyzed.

Results: A total of 918 cases were included in the study. Among those, 159 were called malignant on cytology (17.3%). MOC31, B72.3 and mCEA IHC were used in 69, 81 and 60 cases, respectively (Table 1). The overall sensitivity for MOC31 and mCEA were 47% each

and for B72.3 was 42%. mCEA had the highest NPV followed by B72.3 and MOC31. MOC 31 was positive in 100% of ovarian ADC (O-ADC) (4/4), 60% of lung ADC (L-ADC) (6/10), 60% of breast ADC (B-ADC) (3/5) and 22.2% of pancreatobiliary ADC (PB-ADC) (2/9). B72.3 was positive in 85.7% of O-ADC (6/7), 61.5% of L-ADC (8/13), 28.6% of B-ADC (2/7) and 33.3% of PB-ADC (3/9). mCEA was positive in 50% of PB-ADC (5/10), 50% of O-ADC (1/2), 42% of L-ADC (3/7) and 33.3% of B-ADC (1/3).

Marker	TP	FP	TN	FN	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
MOC31	24	0	18	27	47	100	100	40
B72.3	24	0	25	32	42	100	100	43
mCEA	17	0	19	24	47	100	100	44

Site	MOC31			B72.3			mCEA		
	Positive	Negative	Total	Positive	Negative	Total	Positive	Negative	Total
Lung	6 (60%)	4 (40%)	10	8 (61.5%)	5 (38.5%)	13	3 (42.9%)	4 (57.1%)	7
Pancreatobiliary	2 (22.2%)	7 (77.8%)	9	3 (33.3%)	6 (66.7%)	9	5 (50%)	5 (50%)	10
Colorectal	3 (50%)	3 (50%)	6	2 (50%)	2 (50%)	4	3 (60%)	2 (40%)	5
Breast	3 (60%)	2 (40%)	5	2 (28.6%)	5 (71.4%)	7	1 (33.3%)	2 (66.7%)	3
Ovary	4 (100%)	0 (0%)	4	6 (85.7%)	1 (14.3%)	7	1 (50%)	1 (50%)	2
Other primary sites	6 (35.3%)	11 (64.7%)	17	3 (18.8%)	13 (81.2%)	16	6 (38%)	10 (62%)	16
Benign	0 (0%)	18 (100%)	18	0 (0%)	25 (100%)	25	0 (0%)	19 (100%)	19

Conclusions: The overall specificity and PPV of all three markers were 100%. However, sensitivity and negative predictive values of these markers were below 50% limiting their application as a single marker. The overall sensitivity of MOC31 and mCEA were superior to that of B72.3. When L-ADC or B-ADC are suspected, a combination of MOC31 and B72.3 should be chosen to improve sensitivity. mCEA and B72.3 had a better sensitivity for detection of PB-ADC. For B-ADC, a second IHC in addition to MOC31 could be considered. In recent years the utilization of general markers seems to decrease and are applied primarily in cases with unknown primary prior to the application of lineage specific markers.

359 A Retrospective Review of Our Experience in Targeted Next Generation Sequencing (NGS) on Cytology Centrifuged Supernatants (CCS): Analysis of Performance and Turnaround Time (TAT)

Hamza Gokozan[1], Aparna Harbhajanka[2], Claire Michael[3], Philip Bomeisl[2], Navid Sadri[2]
[1]University Hospitals Cleveland Medical Center, Case Western Reserve University, Cleveland, OH, [2]Cleveland, OH, [3]University Hospitals Cleveland Medical Center, Cleveland, OH

Disclosures: Hamza Gokozan: None; Aparna Harbhajanka: None; Claire Michael: None; Philip Bomeisl: *Consultant*, PathAI; Navid Sadri: None

Background: Molecular testing is an essential step in providing patients with advanced non-small cell lung cancer (NSCLC) the most adequate front-line targeted therapies. We recently implemented targeted NGS on previously discarded cytology centrifuged supernatant (CCS). In this study we evaluated our implementation process to evaluate its performance.

Design: The retrospective review looked at performance and TAT of molecular testing on all cytology NSCLC cases sent for targeted NGS molecular profiling from June 2018 to September 2019. This review captured the 62 cases since implementation of CCS and compared it to 46 cytology FFPE cell block (FFPE-CB) samples which were used prior to implementation of CCS. Since the implementation of this method, NGS testing was not ordered or performed on the CB or concurrent biopsy as a second-line specimen.

Results: The CCS was a more robust sample type. All 62 CCS samples were adequate and successful for both DNA and RNA (100%) analysis. In comparison a lower success rate for RNA analysis (96%) was seen in FFPE-CB cohort as two of the 46 samples failed RNA QC metrics. In addition the use of CCS samples help preserve tissue for other IHC biomarkers. In CCS cohort, there was sufficient sample for all samples for which PD-L1 was ordered (n=47/47: 100%). In comparison the FFPE-CB cohort had a lower success rate (n=42/44: 95%) as testing for PD-L1 IHC was cancelled in two cases due to insufficient sample at time of IHC review. The mean time from date of specimen collection to the NGS resulting was 8.5 ± 1.8 business days with CCS (median: 8; range: 5-13) as compared to 12.2 ± 5.3 business days with FFPE-CB (median: 10; range: 6-27). This difference in turn-around time was mainly due to steps required prior to getting samples to the molecular laboratory: making of the FFPE block, cutting extra unstained slides after initial cytopathologist diagnosis review of H&E, and review for adequacy by AP-molecular service. Furthermore, there was a reduction of 4 hours in extraction time from switching from FFPE-CB to CCS as starting material.

Conclusions: Our results confirm that NGS can be implemented and performed on CCS. The process resulted in improved TAT, improved sample quality, and increase in preservation of tissue for other testing. In addition the decreased processing steps may reduce the risks for potential pre-analytical errors and was associated with cost savings of approximately $22 per case at our institution.

360 Risk of Malignancy Associated with the Diagnostic Categories Proposed by the Papanicolaou Society of Cytopathology for Pancreatobiliary Specimens: An Institutional Experience

Miguel Gonzalez-Mancera[1], Saman Seyed Ahmadian[2], Merce Jorda[1], Carmen Gomez-Fernandez[1], Monica Garcia-Buitrago[3]
[1]University of Miami Miller School of Medicine, Miami, FL, [2]Stanford Health Care, Sunnyvale, CA, [3]University of Miami Miller School of Medicine/Jackson Health System, Miami, FL

Disclosures: Miguel Gonzalez-Mancera: None; Merce Jorda: None; Carmen Gomez-Fernandez: None; Monica Garcia-Buitrago: None

Background: Pancreatic lesions are studied preoperatively via endoscopic ultrasound-guided fine-needle aspiration (EUS-FNA). In 2014, the Papanicolaou Society of Cytopathology proposed a new classification for reporting pancreatobiliary specimens; however, studies of the risk of malignancy (ROM) associated with each category remains limited.

Design: Two-hundred ninety-eight pancreatic FNAs collected from 2010-2019 with available surgical follow-up were retrieved from the University of Miami files. Cytopathological reports were reclassified as non-diagnostic (I), negative for malignancy (II), atypical (III), neoplastic (IVA benign; IVB other), suspicious for malignancy (V), or malignant (VI). Follow up or concurrent surgical diagnoses were correlated with FNA results. The sensitivity, specificity and the risk of malignancy were calculated for each category.

Results: The FNAs were reclassified as follows: 10% (30/298) category I, 15.4% (46/298) category II, 6.7% (20/298) category III, 14% (42/298) category IVB, 6% (18/298) category V, 47.6% (142/298) category VI. The absolute ROM was 20% for category I, 13% category II, 50% category III, 9.5% category IV when intraductal papillary mucinous neoplasm, neuroendocrine neoplasm, and cystic mucinous neoplasm were not characterized as malignant) and 95% when categorized as malignant; 100% category V, and 97% category VI (Table 1). Sensitivity, specificity, positive predictive value and negative predictive value for neoplastic and malignant categories was 96%, 73%, 92% and 86%, respectively.

Cytologic Diagnosis	Absolute risk	Relative Risk
Nondiagnostic (I)	20%	1.5
Negative for malignancy (II)	13%	1
Atypical (III)	50%	3.8
Neoplastic, Other (IV)	9.5%	0.73
Suspicious for malignancy (V)	100%	7.6
Malignant (VI)	97%	7.4

Conclusions: The cytologic categories developed by the Papanicolaou Society of Cytopathology stratify risk of malignancy. Aspirates designated as suspicious for malignancy and malignant had the highest risk for malignancy. This scheme provides useful information to clinicians treating patients with pancreatic lesions.

361 Detection of Severe Dysplasia, Carcinoma In Situ and Squamous Cell Carcinoma in Female Veterans over a Ten Year Period Using Surepath Liquid-Based Cytology and HR-HPV Testing: Success of a Quality Assurance Program

Linda Green, Baylor College of Medicine and VAMC, Bellaire, TX

Disclosures: Linda Green: None

Background: With an increasing emphasis on Veteran Women's Health, we designed a study to see how our VAMC was doing at detecting invasive carcinomas and severe dysplasia/carcinoma in situ (lesions which often progress to carcinoma if untreated). Cervical Cytology is reported to only be sensitive in detecting CIN II-III or Carcinoma in 44-72% of patients in the Private sector. Is it higher in the VA System with higher standards of Quality Assurance and Quality Control?

Design: Histology to cytology correlation statistics (all biopsies, cervical cones and cervical resections), cervical smear diagnosis data and High-risk HPV (HR-HPV) status maintained by the Director of Cytopathology at our VAMC were reviewed from 2008 to 2018. Only patients with Cervical Intraepithelial Neoplasia III or invasive squamous cell carcinoma and adenocarcinoma were used for outcomes. All PAP smears are screened by both Staff Cytotechnologists and Cytopathologists.

Results: From 2008-2018, there were 21,086 cervical smears performed and 184 cases of CIN III or Carcinoma were detected. Of the 6 invasive carcinomas, 2 had no previous pap smear and 4 had their 1st PAP smear show CIN III or Adenocarcinoma. In CIN III, the first cervical smear showed 56 High Grade Squamous Intraepithelial Lesion (HGSIL), 45 Low Grade Squamous Intraepithelial Lesion (LGSIL), 23 LGSIL rule out HGSIL, 44 Atypical Squamous Cells of Undetermined Significance (ASCUS), 8 ASCUS-H and warranting biopsy. Three patients had no VA Pap smear but presented with abnormal cytology in the private sector. High risk Human Papilloma Virus (HR-HPV) was seen in many (Table 1). No patient had a normal cervical smear.

HR-HPV Testing Performance				
Diagnosis	**Number**	**HR-HPV+**	**HR-HPV-**	**Not Done**
HGSIL	56	18	4	34
LGSIL	45	40	2	3
LGSIL rule out HGSIL	23	12	4	7
ASCUS	44	41	3	0
ASCUS-H	8	7	1	0
Adenocarcinoma/HG	2	1	0	1
no smear	6	0	0	0
Total	184	119	14	51

Conclusions: Compared to the private sector, we had a 100% detection rate for CIN III and invasive carcinomas when there was a screening pap smear. None of the patients with CINIII/CIS progressed to an invasive carcinoma because they had been treated for their CIN III/CIS. Although all had an abnormal cytology only 89% of the patients had detected HR-HPV. In summary, a strong QA program can detect cancer and preneoplasia within the VA System that exceeds the private sector.

362 Comparative Cytomorphologic Analysis of Clear Cell Papillary Renal Cell Carcinoma: Distinguishing Diagnostic Features

Brannan Griffin[1], Xiaoqi Lin[2]
[1]Northwestern University Feinberg School of Medicine, Chicago, IL, [2]Northwestern University, Chicago, IL

Disclosures: Brannan Griffin: None; Xiaoqi Lin: None

Background: Clear cell papillary renal cell carcinoma (CCPRCC) shares histomorphologic and immunophenotypic features of both clear cell (CCRCC) and papillary renal cell carcinomas (PRCC); however, morphologic features of CCPRCC have not been described in detail in Cytology specimens. Early recognition and diagnosis of this entity with excellent prognosis would likely optimize patient management. In this investigation, we aim to evaluate the cytomorphologic findings of CCPRCC in Cytology specimens and compare them to features of CCRCC and PRCC.

Design: In this retrospective study, we collected Cytology slides of kidney fine needle aspiration (FNA)/needle core biopsy (NCB) specimens with diagnoses: CCPRCC, CCRCC and PRCC. Smear and/or touch prep slides were reviewed for malignancy cytomorphologic features: cellularity, architecture, background, cell size & shape, and nuclear and cytoplasmic characteristics. NCB slides were reviewed for malignancy histomorphology, immunohistochemical profile, and tissue diagnostic correlation. Data were grouped by diagnosis and compared using Yate's correction test with p-value <0.05 as statistically significant difference.

Results: Our investigation included: 14 cases of CCPRCC, 20 of CCRCC, and 18 of PRCC. Median patient age for CCPRCC was 66 years (range 27-75). Therapy data available for 13 CCPRCC patients showed: 7/13 (54%) nephrectomy, 3/13 (23%) cryo-ablation, and 3/13 (23%) serial MRI with no evidence of metastatic disease. Comparative cytomorphologic features are displayed in Table 1. At rapid on-site evaluation, 12/14 (86%) CCPRCC specimens were deemed adequate for diagnosis; with CCPRCC suspected in only 5/14 (38%) of cases. NCB of CCPRCC showed low-grade malignant cells in nests (57%), tubules (100%), & papillae (79%), frequently in myxohyaline stroma (43%). Immunohistochemical stains demonstrated expression: CK 7 pos (100%); CA-IX pos (100%, cup-like); CD10 pos (50%, reverse cup-like); AMACR neg (70%); CD117 neg (100%).

Table 1: Cytomorphologic Features of Clear Cell Papillary, Clear Cell, and Papillary types of Renal Cell Carcinoma.

	CCPRCC (n=14)	CCRCC (n=20)	PRCC (n=18)	Statistical Significance
Three-dimensional groups	86%	0%	83%	p-value: <0.01
Papillary architecture	57%	0%	100%	p-value: <0.01
Vasculature	Cells organized around	Thin, transversing cells	Cells organized around & thin, transversing cells	
Sheeted pattern	29%	70%	61%	p-value: <0.05
Single cell pattern	71%	100%	39%	p-value: <0.05
Predominant background	Macrophages, Small vacuoles	Small vacuoles, granules	Macrophages, granules, proteinaceous material	
Cell size & shape	Small-medium; Columnar-polygonal	Medium-large; Epithelioid	Small-large; Cuboidal, columnar, & epithelioid	p-value: <0.01; <0.01
Nuclear size & location	Small-medium; Eccentric	Small-large; Eccentric	Small-large; Central	p-value: <0.05; <0.01
Naked nuclei	2%	100%	67%	p-value: <0.01
Nucleoli	Inconspicuous-conspicuous	Conspicuous-prominent	Conspicuous-prominent	p-value: <0.05
Chromatin	Fine-granular	Fine-granular, Coarse	Fine-granular, Coarse	
Nuclear membrane contour	Smooth (100%)	Smooth (25%), Irregular (75%)	Smooth (67%), Irregular (33%)	p-values: <0.01, <0.05
Nuclear grooves	7%	0%	66%	p-value: <0.01
Nuclear inclusions	21%	25%	12%	p-value: <0.05
Cytoplasmic size	Small-large	Medium-large	Small-medium	p-value: <0.01
Clear cytoplasm	77%	95%	44%	
Cytoplasmic vacuoles	Small-medium	Small	Absent-small	
Granular cytoplasm	50%	90%	100%	
Pigmented cytoplasm	2%	0%	83%	p-value: <0.01

Conclusions: We found certain architectural, nuclear, and cytoplasmic features, when observed together, to be diagnostic for CCPRCC in Cytology specimens. Recognition of adequate diagnostic material at on-site assessments is difficult. In cases with NCB, histomorphology and an immunohistochemical panel including CK 7, CA-IX, CD10, and AMACR further establish accurate diagnosis. Distinguishing CCPRCC from more aggressive RCC types in Cytology specimens would enhance clinical management of patients given CCPRCC's low-grade, indolent behavior.

363 Usage of Immunohistochemistry in Body Fluid Cytology: Variation Uncovered by an International Study of over 20,000 Cases from Seven Countries and Four Continents

Elizabeth Grindstaff[1], Stefan Pambuccian[1], Safa Alshaikh[2], Kenzo Hiroshima[3], Anders Hjerpe[4], Deepali Jain[5], Panagiota Mikou[6], Donna Russell[7], Mauro Saieg[8], Christopher VandenBussche[9], Eva Wojcik[1]

[1]Loyola University Medical Center, Maywood, IL, [2]Salmaniya Medical Complex, Hamad Town, Bahrain, [3]Chiba University Graduate School of Medicine, Chiba, Chiba, Japan, [4]Karolinska University Hospital, Huddinge, Sweden, [5]All India Institute of Medical Sciences, New Delhi, India, [6]Laiko Hospital, Athens, Attica Greece, Greece, [7]University of Rochester Medical Center, Rochester, NY, [8]A.C.Camargo Cancer Center, São Paulo, SP, Brazil, [9]Johns Hopkins Hospital, Baltimore, MD

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Background: Body fluid cytology (BFC) is frequently used in the diagnosis and staging of malignancy and is a common specimen received by cytopathologists, accounting for nearly 30% of non-gynecologic cytology (NGC) specimens. Immunohistochemistry (IHC) staining is an important adjunct in BFC interpretation; however there are currently no guidelines in the usage and utilization of IHC in BFC. The purpose of this study was to illustrate the patterns of IHC utilization in BFC in different large academic pathology centers around the world.

Design: We conducted a retrospective study of IHC utilization rates in BFC specimens amongst nine academic institutions in seven countries on four continents. A survey was distributed to these institutions and the data compiled and analyzed using descriptive statistics.

Results: The rates of BFC samples were fairly consistent across all institutions, with an average of 30%, with most of those cases being pleural fluid samples (mean 50%). IHC stains were performed most often on cell block sections (6 institutions), but smears, cytospin preparations and liquid-based cytology preparations were used in one institution each. The usage rates and of IHC was highly variable amongst institutions. When separating usage of IHC by diagnostic category, rates varied from 1-55% (see Table 1). Interestingly, we found no correlation between the IHC usage rate and the rates of "atypical" or "suspicious" diagnoses. As for the rationale of IHC use, diagnosing benign versus malignant was the most common reason to use IHC in all institutions.

We found that the utilization rates of ICH in BFC samples varied significantly amongst different institutions in a multinational analysis. While the use appeared to vary somewhat with the patient population of the institution, with cancer centers reporting a high IHC usage rate, other variable such as resources, and diagnostic “style” most likely play an important role. The use of IHC did not appear to reduce "atypical" or "suspicious" diagnoses, despite the most common rationale for usage being to determine a benign versus malignant diagnosis. This data provides further evidence for the need of guidelines in the most efficient and cost-effective usage of IHC in BFC interpretation.

Figure 1 - 363

Table 1. The average and range for IHC utilization in BFC specimens.

	BFC TYPES				
	% fluid cytology specimens	% pleural	% peritoneal	% pericardial	% pelvic washing
Mean	28	51	28	4	19
Range	7-44	40-70	17-44	0-5	3-32
	DIAGNOSTIC RATES				
	% negative	% positive	% atypical	% suspicious	% unsat
Mean	69	19	5	4	5
Range	56-84	11-27	0-9	1-7	0.5-18
	IHC USE ACCORDING TO FINAL DIAGNOSIS				
	% negative	% positive	% atypical	% suspicious	% unsat
Mean	7	43	53	28	0
Range	0-55	10-90	0-100	0-98	0

Conclusions: We found that the utilization rates of ICH in BFC samples varied significantly amongst different institutions in a multinational analysis. While the use appeared to vary somewhat with the patient population of the institution, with cancer centers reporting a high IHC usage rate, other variable such as resources, and diagnostic “style” most likely play an important role. The use of IHC did not appear to reduce "atypical" or "suspicious" diagnoses, despite the most common rationale for usage being to determine a benign versus malignant diagnosis. This data provides further evidence for the need of guidelines in the most efficient and cost-effective usage of IHC in BFC interpretation.

364 The Role of Fine Needle Aspiration Cytology in Diagnosis Yield and Accuracy of Gynecologic Tumors: Experience of a Large Health Care System

Sean Hacking[1], Nidhi Kataria[2], Baidarbhi Chakraborty[3], Karen Chau[4], Kasturi Das[1], Seema Khutti[5]
[1]Northwell Health, Forest Hills, NY, [2]Northwell Health, Hofstra School of Medicine, Manhasset, NY, [3]Department of Pathology and Laboratory Medicine, Zucker School of Medicine at Hofstra/ Northwell, New Hyde Park, NY, [4]Donald and Barbara Zucker School of Medicine at Hofstra/Northwell, Lake Success, NY, [5]Donald and Barbara Zucker School of Medicine at Hofstra/Northwell, Greenvale, NY

Disclosures: Sean Hacking: None; Nidhi Kataria: None; Baidarbhi Chakraborty: None; Karen Chau: None; Kasturi Das: None; Seema Khutti: None

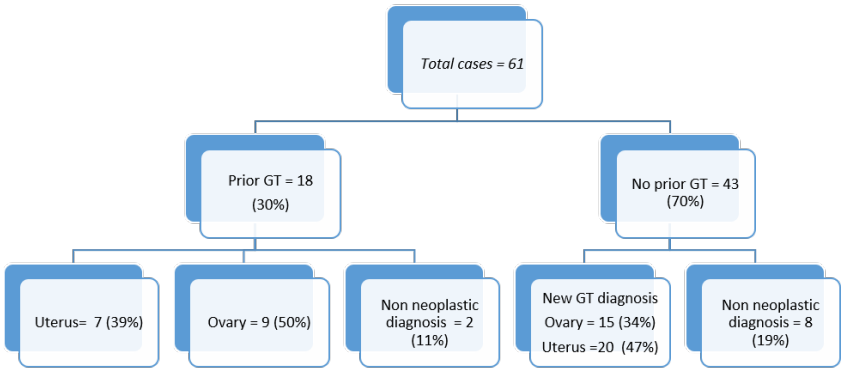
Background: *There is limited literature available regarding the role of fine needle aspiration cytology (FNAC) in the diagnosis of gynecologic tumors (GT), despite the fact that many GT tumors present as abdominal/peritoneal tumors in advanced malignancy. The aim of this current study was to evaluate the role of FNA in the diagnostic yield, accuracy and triaging of GT.*

Design: A retrospective review of our pathology database for cases with a cytological GT diagnosis, over four-year period (2016-2019) was conducted. The cases comprised of either FNA only (FO), FNA and Core biopsy (FCB) or Touch prep and core biopsy (TCB). Clinical/pathological data including site, immunohistochemistry treatment modalities and prior history of GT were noted.

Results: Our cohort included 61 patients, out of which, there were 24, 22 and 15 cases of FO, FCB and TCP respectively. The common sites of FNAC were peritoneum (33), pelvis (19), abdominal wall (5), retroperitoneum (2), vagina (1) and peri-spinal area (1). The concordance rates and diagnostic accuracy for FO, FCB and TCB are summarized in (Table.1). All cases underwent rapid on-site evaluation (ROSE) for adequacy except 5/6 cases. The most useful immunohistochemical stains utilized to achieve diagnosis were PAX-8, WT1 and CK7 which were ordered, in 42, 24 and 22 of 61 cases respectively. Out of the 23 cases with surgical follow up, 17 (74%) were managed with chemo-radiation based on their cytological diagnosis. Of the 38 cases without surgical follow up, 13 were treated with chemo-radiation; 7 patients were deceased 2 patients refused treatment and 16 lost follow-up.

	Non-Diagnostic	Negative	Atypical	Suspicious	Positive	Total
FNA only	1	3	1	0	19	24
FU	1	2	1	0	6	10
Concordant	0/1	1/2	0/1	NA	6/6	7/10
FNA and core	1	1	0	0	20	22
FU	0	0	0	0	8	8
Concordant	NA	NA	NA	NA	8/8	8/8
TP and core	0	0	0	0	15	15
FU	0	0	0	0	5	5
Concordant	NA	NA	NA	NA	5/5	5/5

Figure 1 - 364



Conclusions: All three methods of procurement had 100% concordance when the case was deemed positive. There were no non-diagnostic cases in TCB category (sample size is small for any statistical significance between the 3 methods). FO and FCB procedure was plagued by "atypical" or "non-diagnostic" category. Availability of ROSE overcame any indeterminate interpretation and is imperative in appropriate triaging of patient for further management. About 50 %(30/61) received chemotherapy or radiation based on cytologic diagnosis only. Hence, cytologic diagnosis plays vital role in prompt initiation of oncological treatment.

365 A Comparison of the Frequency of 'Atypia of Undetermined Significance' Interpretations for Thyroid Fine-Needle Aspirations over a Two Year Period

Sarah Hackman[1], David Kim[2], Diane Avery[3], Alia Nazarullah[2], Daniel Mais[1]
[1]UT Health San Antonio, San Antonio, TX, [2]The University of Texas Health Science Center at San Antonio, San Antonio, TX, [3]University Hospital, San Antonio, TX

Disclosures: Sarah Hackman: None; David Kim: None; Diane Avery: None; Alia Nazarullah: None; Daniel Mais: None

Background: The Bethesda System for Reporting Thyroid Cytopathology (TBSRTC) outlines six distinct diagnostic categories for reporting thyroid cytopathology. The category of "Atypia of Undetermined Significance" (AUS) or "Follicular Lesion of Undetermined Significance" (FLUS) is used for specimens that show architectural and/or nuclear atypia that is interpreted as between Benign and Suspicious for Malignancy categories. TBSRTC predicted the AUS category to represent no more than 7% of diagnoses; however, in practice there is high interobserver variability, with reported individual rates ranging from 2.5-28.6%. The purpose of this study was to examine the AUS rates for new-in-practice and senior pathologists and determined how these rates changed with increasing experience.

Design: We reviewed thyroid fine-needle aspiration (FNA) diagnoses for three pathologists not boarded in cytopathology over their first and second full years of interpreting thyroid cytopathology. We compared the percent of AUS cases for the first two years of practice to the rate of AUS diagnoses for a senior pathologist with several years of experience with thyroid FNAs. AUS data was also examined for conventional smears (CS) and ThinPrep (TP) to see if the slide preparation technique impacted AUS rates.

Results: A total of 807 thyroid FNA diagnoses were retrospectively reviewed, of which 172 were diagnosed as AUS (21.3%). The percentage of cases diagnosed as AUS for junior pathologists ranged from 19.6-30.8% in their first year of practice to 19.2-42.6% in their second year of practice (Table 1). The senior pathologist AUS rate for two years remained relatively stable at 14.7% and 15.5%, regardless of slide preparation technique. Junior pathologists diagnosed AUS more frequently on CS versus TP, 29% and 25%, respectively.

Table 1. Comparison of rates of AUS by experience level

Level of experience	YEAR 1	YEAR 2
Junior pathologist 1	29/94 (30.8%)	26/61 (42.6%)
Junior pathologist 2	20/102 (19.6%)	10/52 (19.2%)
Junior pathologist 3	12/47 (25.5%)	22/99 (22.2%)
Junior average	**61/243 (25.1%)**	**58/212 (27.3%)**
Senior pathologist	29/197 (14.7)	24/155 (15.5%)

Conclusions: The rates of AUS for junior pathologists are widely variable and remain higher for the first two years of practice compared to the relatively constant rate of AUS diagnoses for a senior pathologist. Although the average rates of AUS for junior pathologists in this study are on the higher side of the ranges reported in the literature, even the senior pathologist AUS percentage is double the goal outlined

in TBSRTC. The wide variation in reported AUS diagnoses across individuals and institutions may be influencing the idealized 5-15% expected malignancy rate in this category. Future morphologic or molecular subcategorization of this category may be required to properly risk stratify patients.

366 Tiny But Mighty: A Successful Use of Discarded Cytocentrifuged Specimen of Bile Duct Brushings with Application of Next Generation Sequencing to Increase Sensitivity of Cytological Diagnosis.

Aparna Harbhajanka[1], Claire Michael[2], Nafiseh Janaki[3], Hamza Gokozan[4], Jay Wasman[2], Philip Bomeisl[1], Jennifer Yoest[5], Navid Sadri[1]

[1]Cleveland, OH, [2]University Hospitals Cleveland Medical Center, Cleveland, OH, [3]Brigham and Women's Hospital, Harvard Medical School, Boston, MA, [4]University Hospitals Cleveland Medical Center, Case Western Reserve University, Cleveland, OH, [5]UPMC, Pittsburgh, PA

Disclosures: Aparna Harbhajanka: None; Claire Michael: None; Nafiseh Janaki: None; Hamza Gokozan: None; Philip Bomeisl: *Consultant*, PathAi; Navid Sadri: None

Background: Bile duct brushing (BDB) is used to evaluate pancreatobiliary lesions as it widely samples lesions with a low complication rate. Cytological evaluation of BDB is a specific but insensitive test. There is limited literature on the use of post-cytocentrifuged specimens, which are usually discarded, for next-generation sequencing (NGS) as an adjunct to cytological diagnosis of BDB. In this study we investigate if molecular analysis by NGS of post-cytocentrifuged specimen improves the sensitivity of diagnosis.

Design: Post cytocentrifuged (PCC) specimen from 100 consecutive BDB specimens spanning 93 unique patients were retained. DNA was extracted and mutational analysis was performed agnostic of morphologic or clinical findings. Each BDB specimen was characterized as negative, atypical/suspicious or positive based on morphological analysis by trained cytopathologists. Performance characteristics for mutational profiling and morphological analysis were calculated on the basis of clinicopathologic follow-up.

Results: The 100 cases were classified as 44 malignant, 51 benign and 5 indeterminate based on clinicopathologic follow-up (Table.1). Based on morphologic analysis of cytology, the cases were classified as 16 malignant, 56 benign, and 28 atypical/suspicious (Fig.1). Out of 24 cases which showed atypical cells on cytology and for which adequate clinical follow up was available, NGS revealed mutations in 19 cases (Table 1). Morphologic analysis of cytology showed a sensitivity of 36% and a specificity of 100 % if atypical cases were considered negative (or not malignant). NGS revealed oncogenic alterations in 93% cases of malignant cases based on clinicopathologic follow-up. The most common alterations where in *KRAS* and *TP53*, observed in 71% and 48% of malignant cases respectively (Fig.2). No alterations were observed in the 51 benign cases based on clinicopathologic follow-up. Addition of NGS analysis to morphologic analysis of cytology increased the sensitivity to 93% and maintained specificity at 100%.

NGS * Clinical diagnosis * Cytological diagnosis

Cytological diagnosis			Clinical diagnosis						Total
			Benign	Pancreatic ductal carcinoma	Cholangiocarcinoma	Gall bladder carcinoma	Indeterminate	ampullary carcinoma	
Negative	NGS	Negative	48	1	1		0		50
		Positive	0	3	2		1		6
	Total		48	4	3		1		56
Atypical	NGS	Negative	3	1	0		1	1	6
		Positive	0	16	1		3	2	22
	Total		3	17	1		4	3	28
Adenocarcinoma	NGS	Positive		10	5	1			16
	Total			10	5	1			16
Total	NGS	Negative	51	2	1	0	1	1	56
		Positive	0	29	8	1	4	2	44
	Total		51	31	9	1	5	3	100

Figure 1 - 366

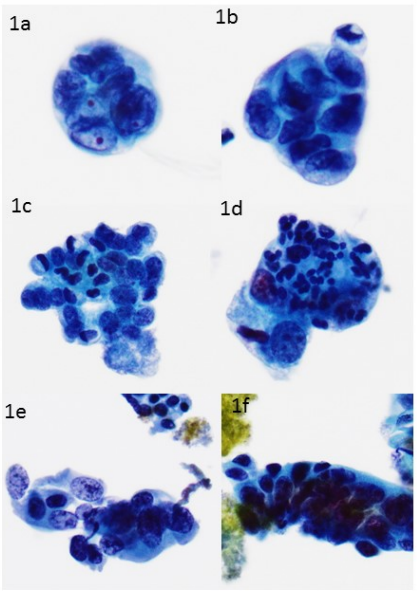


Figure 1. a-b, Representative atypical case . Morphological images of rare atypical cells in case that had *KRAS* and *TP53* mutation on mutation analysis and patient had pancreatic adenocarcinoma on follow-up.(Pap stain, 600x); c-d, Representative atypical case favor reactive. *KRAS* and *TP53* mutation on mutation analysis and patient had pancreatic adenocarcinoma on follow-up. (Pap stain, 400x);

Figure 2 - 366

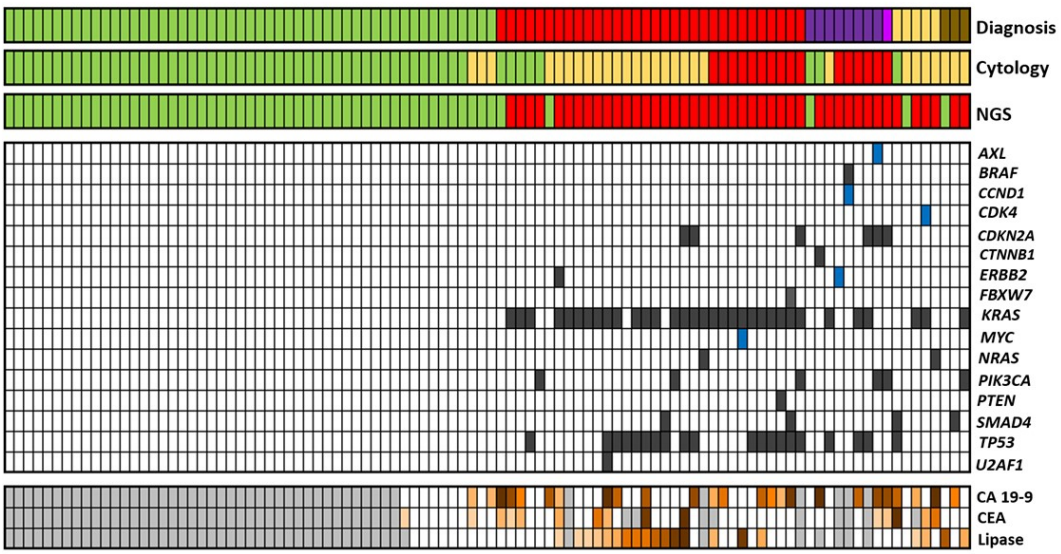


Figure 2. Heatmap depicting correlative findings of cytological examination of bile duct brushings and individual genomic alterations from NGS testing with corresponding patient follow up. Each column represents a different case. Each row depicts another variable being compared. 'Diagnosis' row depicts clinical diagnosis: benign (green, n=51), pancreatic adenocarcinoma (red, n=31), cholangiocarcinoma (purple, n=9), gallbladder cancer (magenta, n=1), atypical with no further follow-up (yellow, n=5), and ampullary or duodenal mass considered malignant (brown, n=3). 'Cytology' row depicts morphological diagnosis: benign (green, n=56), atypical (yellow, n=28), and malignant (red, n=16). The 'NGS' row depicts molecular analysis diagnosis based on NGS findings: benign (green, n=56) and malignant (red, n=44). For each gene row, the gray box indicates a mutations and blue box indicates a gene amplification was observed. For each serum biomarker, values above normal range [CA 19-9 (>30 U/mL), CEA (>2.5U/ml), Lipase (>82 U)] are shown in tan to brown (with increasing levels). Cases for which serum biomarkers are not available are shown in gray.

Conclusions: This study provides evidence for the utility of using NGS PCC specimen to increase the sensitivity of BDB cytology samples, although studies with larger cohorts are needed to verify these findings.

367 Comparison of Sensitivity and Specificity for Hybrid Capture 2 and Cobas 4800 in Detecting HPV for CIN2+ Patients

Aparna Harbhajanka[1], Mingfei Yan[2], Philip Bomeisl[1], Claire Michael[3]
[1]Cleveland, OH, [2]Case Western Reserve University/University Hospitals Cleveland Medical Center, Cleveland, OH, [3]University Hospitals Cleveland Medical Center, Cleveland, OH

Disclosures: Aparna Harbhajanka: None; Mingfei Yan: None; Philip Bomeisl: *Consultant*, PathAi; Claire Michael: None

Background: The Hybrid Capture 2 high-risk HPV test (HC2) and Cobas 4800 HPV test (Cobas 4800) are two commonly used molecular methods for detecting high-risk HPV (HR-HPV) strains in clinical practice, which are different in both molecular mechanisms and target genes. Since our institute changed the system from HC2 test to Cobas 4800 test in 2015, we found it is essentially important to compare the two tests and correlate with histological outcomes.

Design: In this retrospective study, data from 7440 Pap tests for HR-HPV testing with histological follow up was gathered and analyzed, which includes 4637 cases analyzed by HC2 from January 2012 to September 2015 and 2803 cases analyzed by Cobas 4800 Test from September 2015 to December 2017. Then we used surgical follow up of cervical intraepithelial neoplasia 2/3 (CIN2+) as the clinical outcome for Pap tests and compared the sensitivity and specificity of both tests during the two time periods

Results: The HR-HPV positivity rates for HC2 and Cobas 4800 were 53.1% (2461/4637) and 69.8% (1957/2803), respectively. On histological follow up, CIN2+ was diagnosed in 7.6% cases (355/4637) before September 2015 compared to 9.7% cases (273/2803) after September 2015 (Table 1). The test sensitivity for CIN2+ was 91.5% (312/341) by HC2 and 93.9% (248/264) by Cobas 4800. The test specificity for CIN2+ was significantly higher by HC2 (63.8%, 1797/2815) than Cobas 4800 (38.9%, 612/1572) with p-value <0.001.

Table 1. HPV results by Hybrid capture test and Cobas test and histological diagnosis.

Histological diagnosis		Hybrid capture 2	Cobas test	Total	P value
Negative/Benign	HPV negative	1797 (63.8%)	52(23.3%)	2400 (54.9%)	<0.0001
	HPV positive	1018 (36.2%)	960 (61.1%)	1938 (45.1%)	
	Total	2815 (64.2%)	1572 (35.8%)	4387	
CIN2+	HPV negative	29(8.5%)	16(6.0%)	45 (7.4%)	0.25
	HPV positive	312(91.5%)	248(93.6%)	560 (92.4%)	
	Total	341 (56.3%)	264 (43.7%)	605	

Conclusions: The sensitivity and detection rates of CIN2+ with Cobas 4800 was slightly higher though not significantly higher than HC2, while the specificity of Cobas 4800 was significantly lower than HC2. Future studies are required to confirm these findings.

368 Prognostic Significance of the Single Cell Pattern in Fine-Needle Aspirations Diagnostic of Pancreatic Ductal Adenocarcinoma

Christopher Hartley[1], Wegahta Weldemichael[1]
[1]Medical College of Wisconsin, Milwaukee, WI

Disclosures: Christopher Hartley: None; Wegahta Weldemichael: None

Background: The initial diagnosis of pancreatic ductal adenocarcinoma (PDAC) is frequently made via fine-needle aspiration (FNA). FNA smears exert physical strain on epithelial clusters, and represent a test of cohesion. Some tumors resist the strain and remain in clusters, while others exhibit decohesion, with single cells shedding off of the cellular groups. The single cell pattern (SCP) is a correlate of the epithelial-mesenchymal transition (EMT), and is a finding well-described in cytology specimens diagnostic of PDAC. To our knowledge, no studies have specifically addressed the prognostic significance of SCP in PDAC. Our aim was to correlate SCP in FNAs diagnostic of PDAC with overall survival and with clinicopathologic features at resection.

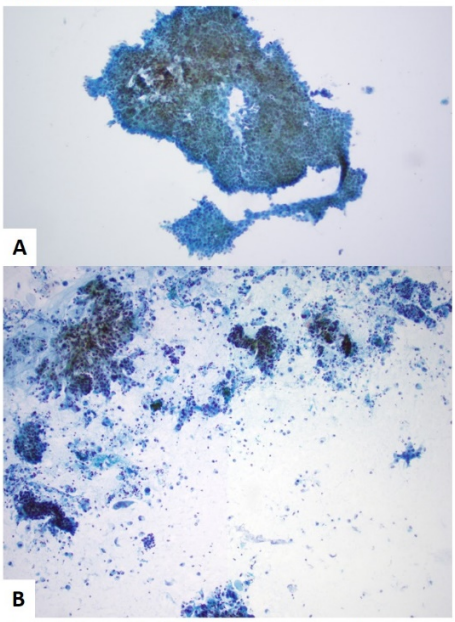
Design: 69 consecutive FNAs diagnostic of PDAC were identified and retrieved from the pathology archives. Of these, 33 patients went on to have resections and all patients had received neoadjuvant chemotherapy and/or radiation. Slides were reviewed and the most cellular smears were selected and evaluated for the SCP. SCP was defined as single cells readily visible separate from clusters at 100x (Figure 1). Presence of SCP was compared to clinicopathologic features gathered from chart review, including 32 cases where the tumors were resectable. ypN and ypT stages are presented according to the AJCC 8th edition criteria.

Results: The SCP was observed in 43/69 (62%) FNAs diagnostic of PDAC. SCP was significantly correlated with worse survival (p=0.04, Figure 2). SCP was not significantly correlated with ypT stage ypN stage, tumor size, perineural invasion, or lymphovascular invasion at resection (Table 1).

Table 1: Summary of Clinicopathologic Features in Correlation with the Single Cell Pattern					
	SCP present (n=43)	**SCP absent (n=26)**	**All Cases**	**Statistics for SCP, presence vs. absence**	**Statistical Method**
Median survival, days (95% CI)	335 (170-890)	829 (543-NA)	596 (308-1018)	**p=0.04**	Log rank test (Kaplan-Meier curve, Figure 2)
Not Resected (n=36)	24/43 (56%)	12/26 (46%)	36/69(52%)	p=0.46	Fisher exact test
ypT stage (n=33)	T1(6);T2(11);T3(2)	T1(2);T2(10);T3(2)	T1(8);T2(21);T3(4)	p=0.58	Fisher exact test
ypN stage (n=33)	N0(14);yN1(2);yN2(3)	N0(9);N1(2);N2(3)	N0(23);N1(4);N2(6)	p=0.87	Fisher exact test
Resection Tumor size (cm), mean±SD(range) (n=33)	3.1±1.6(1.5-8.5)	3.2±1.3(1.1-6.5)	3.1±1.3(0.2-7.5)	p=0.87	t-test
LVI at Resection (n=33)	2/19(11%)	2/14(14%)	4/33(12%)	p=1.0	Fisher exact test
PNI at Resection (n=33)	8/19(42%)	7/14(50%)	15/33(45%)	p=0.73	Fisher exact test
Legend: SCP=single cell pattern; LN= lymph node, LVI= lymphovascular invasion; PNI=Perineural invasion; FNA=fine-needle aspiration; SD=standard deviation; OR=odds ratio; CI=confidence interval.					

Figure 1 - 368

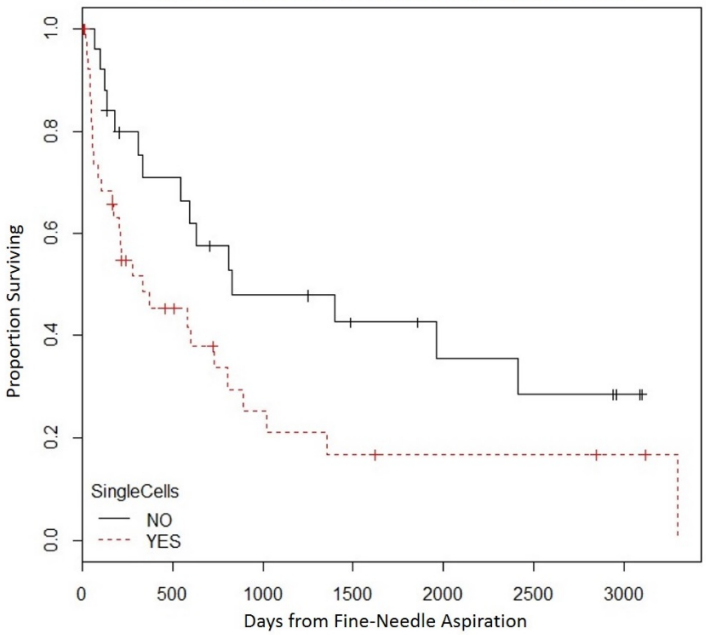
Figure 1: Single Cell Pattern in Pancreatic Ductal Adenocarcinoma Fine-Needle Aspirations



Legend: Pancreatic Ductal Adenocarcinoma showing no single cells (A) and a prominent single cell pattern (B). Pap stain X100 magnification.

Figure 2 - 368

Figure 2: Overall Survival Stratified by Single Cell Pattern



Conclusions: The SCP is readily appreciated in a majority FNAs diagnostic of PDAC, and is significantly correlated with worse survival. SCP may indicate a more aggressive phenotype in PDAC independent of other prognostic features. A larger cohort with dedicated second review of resections will be pursued to further evaluate the significance of the SCP in FNAs diagnostic of PDAC.

369 Next Generation NanoString Multiplex Fusion Sarcoma Assay Performance in DiffQuik and Papanicolaou-Stained Smears of Sarcomas

Zeinab Hasan[1], Hasanain Hasan[2], Shadi Qasem[3], Dana Richards[4], Shulin Zhang[5], Therese Bocklage[4]
[1]The University of Tennessee Health Science Center, Memphis, TN, [2]NYU Langone, New York City, NY, [3]University of Kentucky Healthcare, Lexington, KY, [4]University of Kentucky College of Medicine, Lexington, KY, [5]UKY COM Department of Pathology and Laboratory Medicine, Lexington, KY

Disclosures: Zeinab Hasan: None; Hasanain Hasan: None; Dana Richards: None; Shulin Zhang: None; Therese Bocklage: None

Background: About 50% of sarcomas contain a specific gene fusion. These sarcomas may be difficult to distinguish on standard cytology review and may remain diagnostically problematic after ancillary immunohistochemical staining. Mesenchymal tumors, including fusion positive sarcomas, are increasingly being biopsied by core needles and fine needles. To diagnose a specific fusion sarcoma on a cytology specimen, a multiplex assay could offer advantages over single fusion assays due to 1) higher success rate with a limited tumor sample, 2) lower cost and 3) faster turn-around-time. Recently, a NanoString nCounter (NanoString Inc., Seattle) multiplex fusion sarcoma assay was developed and tested successfully in resection specimens and core biopsies. We evaluated this multiplex fusion assay in sarcomas collected as direct smears, fixed in 95% ethanol and stained with the DiffQuik™ or Pap methods.

Design: After IRB approval, we selected current and archived immediate smears of mesenchymal tumors with paired alternate genetic test results (by PCR, karyotype or FISH analyses). We isolated RNA using a standard Qiagen™ kit method. Twelve microliter sample aliquots were combined with an oligonucleotide fusion probe mix and processed on the NanoString Sprint instrument. The probe mix detects 174 fusions in 25 sarcomas and was designed by Chang and Ng et al. For four specimens, matched resection specimens or core biopsies (FFPE samples) were also tested.

Results: Forty one of 53 cases (77%) were successfully tested including archived smears dating to 2012. Cases included Ewing sarcomas (ES), synovial sarcomas (Fig. 1), undifferentiated pleomorphic sarcomas (anticipated to be negative), alveolar rhabdomyosarcomas (Fig. 2) and a mesenchymal chondrosarcoma. Compared with paired FFPE samples, all cytology smears yielded accurate results (n = 4 pairs). Cases with insufficient RNA quantity or quality unable to be tested included a tenosynovial giant cell tumor and aneurysmal bone cysts. Cases failing the test despite sufficient RNA included 95% necrotic ES and non-necrotic but hypocellular myxoid liposarcoma.

Figure 1 - 369

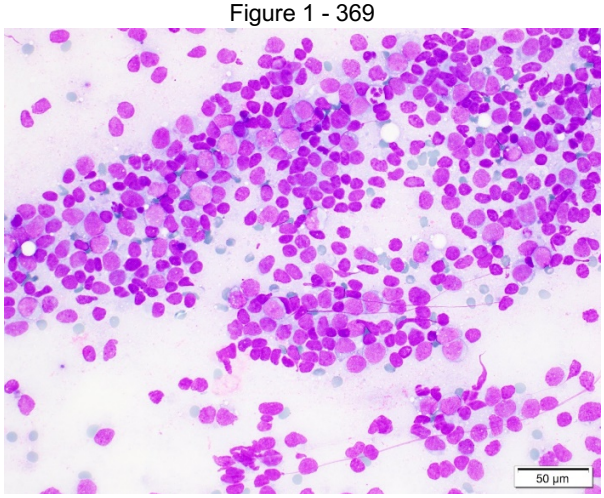
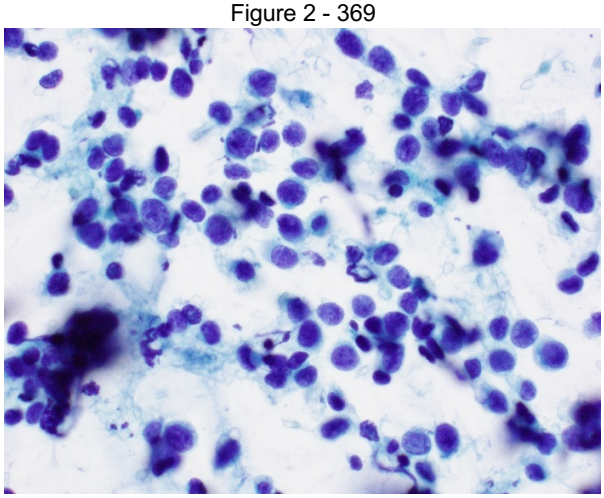


Figure 2 - 369



Conclusions: The multiplex fusion sarcoma assay accurately detects diagnostic fusions in *cellular* smears of mesenchymal tumors. The assay requires adequate RNA quality (%DV200 >5, preferably >15) and concentration > 7 ng/ul (higher with low %DV200). We are now validating the assay for clinical use, as it is relatively fast and low cost but most importantly, feasible using direct smears.

370 ATRX/DAXX Status on Diagnostic FNA Informs Progression Free Survival Prognostication in Pancreatic Neuroendocrine Tumors

Matthew Hedberg[1], Cory Bernadt[2]
[1]*Washington University School of Medicine, St. Louis, MO,* [2]*Washington University, St. Louis, MO*

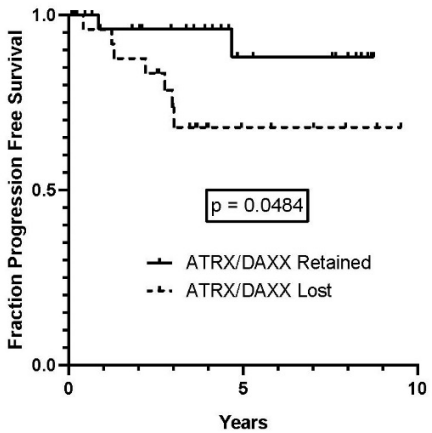
Disclosures: Matthew Hedberg: None; Cory Bernadt: None

Background: Pancreatic neuroendocrine tumors (PanNETs) represent the second most common neoplasm of the pancreas. Their clinical behavior is unpredictable. ≈50% of patients develop metastatic disease; while others have comparably indolent tumors. Endoscopic ultrasound-guided fine-needle aspiration (FNA) biopsy is widely used for the diagnosis of pancreatic lesions including PanNETs. Sequencing has identified mutations in α-thalassemia/mental retardation X-linked (*ATRX*) and death domain-associated protein (*DAXX*) genes in 20-54% of PanNETs; which are associated with loss of nuclear expression of their respective proteins by immunohistochemistry (IHC). *ATRX/DAXX* loss by IHC in PanNET resection specimens is associated with larger tumors, higher stage and decreased survival. Recent studies have shown good correlation between *ATRX/DAXX* status by IHC on FNA biopsies and paired resection specimens, but were not equipped to interrogate clinical outcomes. The purpose of this study is to assess the clinical implication(s) of *ATRX/DAXX* status by IHC on FNA biopsy.

Design: Electronic medical record review identified 55 patients with PanNETs diagnosed by FNA biopsy for whom significant clinical follow-up was available (average: 3.9 years). *ATRX/DAXX* status was assessed by IHC on the diagnostic FNA biopsy cell block by two blinded pathologists. If ≥50% of the observed tumor cells showed nuclear staining, *ATRX/DAXX* was considered retained. Otherwise, it was considered lost. *ATRX/DAXX* status was then correlated with clinical outcomes and staging criteria on subsequent paired resection specimens.

Results: *ATRX/DAXX* loss by IHC on FNA biopsy was correlated with, on resection, significantly higher tumor grade (p = 0.0461), N stage (p = 0.0351) and Ki-67 index (p = 0.0069). It was also associated with higher T Stage, M Stage, clinical stage, LVSI and PNI; though these differences did not achieve statistical significance. Further, *ATRX/DAXX* loss by IHC on FNA biopsy was found to be correlated with significantly worse progression free survival (67.9% vs 88.0%, p = 0.0484) [Fig 1].

Figure 1 - 370



Conclusions: These retrospective data suggest that *ATRX/DAXX* status by IHC on diagnostic FNA biopsy correlates with high grade tumor features and may be a prognostic biomarker that can be applied prior to surgical intervention in PanNETs. Thereby informing treatment decisions in patients who are borderline or high risk surgical candidates. Further study, including a prospective clinical trial with multivariate regression modeling is warranted.

371 Cytology Adds Value to Monoclonal Antibody DAS-1 Testing for Detection of High-Risk Pancreatic Cysts

Amin Heidarian[1], Koushik Das[2], Mari Mino-Kenudson[3], Carlos Fernandez-del Castillo[3], Martha Pitman[4]
[1]Massachusetts General Hospital, Harvard Medical School, Cambridge, MA, [2]Washington University, St. Louis, MO, [3]Massachusetts General Hospital, Boston, MA, [4]Massachusetts General Hospital, Harvard Medical School, Boston, MA

Disclosures: Amin Heidarian: None; Koushik Das: None; Mari Mino-Kenudson: None; Carlos Fernandez-del Castillo: None; Martha Pitman: None

Background: Determining the risk of malignancy in pancreatic cysts (PC) is a clinical and diagnostic challenge. DAS-1 monoclonal antibody (mAb) has recently been shown to have a high sensitivity, specificity and accuracy in detecting cysts at high-risk (HR) for malignancy. DAS-1 mAb test detects HR mucinous cysts with high-grade dysplasia, invasive carcinoma and/or any intestinal-type epithelium. Correlation of mAb DAS-1 testing of PC fluids with specific cytomorphological findings has not been evaluated.

Design: We correlated pre-operative EUS-FNA cytology findings of PC with subsequent mAb DAS-1 test results and resection histology in 26 PCs from our institution included in the original study of mAb Das-1 testing of PC fluid.[1] Histologically, HR mucinous cystic neoplasms (MCN) included the ones with high-grade dysplasia (HGD) or invasive carcinoma. The HR intraductal papillary mucinous neoplasms (IPMN) included intestinal-type IPMNs with intermediate-grade dysplasia (IGD), and HGD or invasive carcinoma of any epithelial type. HR PCs by cytology followed the Papanicolaou Society of Cytopathology Terminology system and included cysts with high-grade epithelial atypia in a mucinous cyst, oncocytic IPMN, and cystic neuroendocrine tumors.

Results: There were 19 IPMNs, 4 MCNs, 2 serous cystadenomas (SCA) and 1 pancreatic neuroendocrine tumor (PanNET).

In 17 cases (65.38%), cytology and DAS-1 correlated with histology of an HR PC. There were 2 (7.69%) DAS-1 negative HR PCs diagnosed by cytology. Five (19.23%) DAS-1 positive HR PCs had mucin only or cells with low-grade dysplasia (LGD) on cytology. Two DAS-1 positive HR PCs had non-diagnostic cytology (Table 1).

HR oncocytic IPMNs and neuroendocrine tumors are not detected by mAb DAS-1 test in this cohort. When either cytology or mAb DAS-1 is positive, accuracy for detection of an HR PC is highest (Table 2).

Table 1: Comparison of histological evaluation with cytology findings and mAb DAS-1 test. IPMN, intraductal papillary mucinous neoplasm; MCN, mucinous cystic neoplasm; PanNET, pancreatic neuroendocrine tumor; SCA, serous cystadenoma; HR, high risk; LR, low risk; HGD, high-grade dysplasia; LGD, low-grade dysplasia.

Number of Cases	Percentage	Risk of Malignancy	Histological Result	Cytology Result	DAS-1 mAb Result
10	38.46 %	HR	IPMN/MCN – HGD/invasion	HGD	+
2	7.69 %	HR	IPMN/MCN - HGD	Non-diagnostic	+
1	3.84 %	HR	Oncocytic-IPMN - HGD	HGD	-
1	3.84 %	HR	PanNET	PanNET	-
4	15.38 %	HR	IPMN - HGD	LGD	+
5	19.23 %	LR	IPMN/MCN - LGD	LGD	-
1	3.84 %	LR	IPMN - LGD	HGD	-
2	7.69 %	LR	SCA	SCA	-

Figure 1 - 371

Table 2: Sensitivity, specificity and accuracy.

Method	Sensitivity, %	Specificity, %	Accuracy, %
DAS-1 mAb +	88.88	100	92.30
Cytology	66.66	87.50	73.07
DAS-1 mAb +/cytology combined ("or" manner)	96.30	87.50	96.15
DAS-1 mAb +/cytology combined ("and" manner)	59.24	100	65.38

Conclusions: The mAb Das-1 is a sensitive and specific biomarker for detecting HR mucinous PCs. Adding cytology to DAS-1 mAb testing improves the sensitivity for the detection of non-mucinous HR PC. Together, cytology with DAS-1 mAb testing is more accurate than either one alone.

Reference:

1. Das, K.K., et al., *Cross Validation of the Monoclonal Antibody Das-1 in Identification of High-Risk Mucinous Pancreatic Cystic Lesions.* Gastroenterology, 2019. **157**(3): p. 720-730 e2.

372 Implementation of the Milan System for Reporting Salivary Gland Cytopathology (MSRSGC): A Cytohistologic Correlation Study from a Large Academic Medical Center

Issa Hindi[1], Oliver Szeto[2], Osvaldo Hernandez[3], Wei Sun[4], Aylin Simsir[5], Tamar Brandler[6]
[1]NYU Langone Medical Center, New York, NY, [2]NYU Langone Health, New York, NY, [3]New York, NY, [4]New York University Langone Medical Center, New York, NY, [5]New York University School of Medicine, Edison, NJ, [6]NYU Langone Health, Highland Park, NJ

Disclosures: Issa Hindi: None; Oliver Szeto: None; Osvaldo Hernandez: None; Wei Sun: None; Aylin Simsir: None; Tamar Brandler: None

Background: Salivary gland neoplasms are rare and the majority are benign with only 20% displaying malignancy. Fine needle aspiration (FNA) plays an essential role in the initial evaluation of salivary gland lesions by providing a pre-operative diagnosis to determine appropriate management. Recently, a tiered classification system known as the Milan System for reporting Salivary Gland cytopathology (MSRSGC) has been published. This system formalizes diagnostic categories with related malignancy risk, recommended clinical therapy and follow-up. Our study aims to compare sensitivity, specificity and risk of malignancy (ROM) between the MSRSGC and the original FNA cytology diagnostic categories used at our institution to determine if the MSRSGC offers added benefit.

Design: Salivary gland cytology slides from subjects with final surgical pathology resections from 11/2016-06/2019 were blindly reviewed and classified according to the MSRSGC. MSRSGC diagnoses were correlated with surgical pathology diagnoses and compared to the original cytology diagnostic categories. Sensitivity, specificity and ROM of diagnostic categories were calculated for both systems.

Results: Follow-up histopathology was available for 101 patients with salivary gland lesions. The MSRSGC had a sensitivity of 69.0% and a specificity of 92.9%. The original classification system had a sensitivity of 75.0% and a specificity of 89.9%. ROM for MSRSGC categories and original diagnostic categories are given in Table 1 and listed side by side to reflect distribution of cases in each system.

Table 1: Malignancy risk associated with MSRSGC and original classification categories

Original Classification Diagnostic Category	Original Classification Malignancy Risk	MSRSGC Diagnostic Category	MSRSGC Malignancy Risk	
*Negative for malignancy	**25.0%** (6/24)	*I. Non-diagnostic	**20.0%** (1/5)	
		*II. Non-neoplastic	**50.0%** (3/6)	
Atypical	**0.0%** (0/3)	III. AUS	**30.0%** (3/10)	
**Positive for neoplasm	**10.6%** (6/56)	**IVA. Neoplasm: Benign	**6.8%** (3/44)	¥**8.5%** (5/59)
		IVB. Neoplasm: SUMP	**13.3%** (2/15)	
Suspicious for malignancy	**50.0%** (1/2)	V. Suspicious for malignancy	**60.0%** (3/5)	
Positive for malignancy	**100.0%** (16/16)	VI. Malignant	**81.2%** (13/16)	

Abbreviations: AUS, atypia of undetermined significance; SUMP, salivary gland neoplasm of uncertain malignant potential
*Most cases originally classified as negative for malignancy fell into MSRSGC category I and II
**Most cases originally classified as positive for neoplasm fell into MSRSGC category IVA and IVB
¥ Combined risk for MSRSGC category IVA and IVB

Conclusions: Performance of the MSRSGC was comparable to that of the original classification system in the majority of cases. Both systems had a similar sensitivity, specificity and ROM in the equivalent categories. The single “non-diagnostic” and the three “non-neoplastic” cases under MSRSGC that showed histopathologic evidence of malignancy were called “negative for malignancy” in the original classification showing lack of cytohistologic correlation for both systems due to sampling errors. Two of the three cases classified as “atypia of undetermined significance” under the MSRSGC were originally classified as “negative for malignancy”. Our findings suggest that traditional diagnostic classification methods for salivary gland cytopathology already established at an institution can perform as well as the MSRSGC in relaying the appropriate diagnostic information, undermining the need for transition to a new classification system.

373 Immunohistochemical Cytology-Histology Correlation in Tubo-Ovarian Neoplasms: Can Cell Blocks Substitute For Tissue?

Anjelica Hodgson[1], Sharon Nofech-Mozes[2], Joerg Schwock[3], Jelena Mirkovic[2], Zeina Ghorab[2]
[1]University of Toronto, Toronto, ON, [2]Sunnybrook Health Sciences Centre, University of Toronto, Toronto, ON, [3]University Health Network, University of Toronto, Toronto, ON

Disclosures: Anjelica Hodgson: None; Sharon Nofech-Mozes: None; Joerg Schwock: None; Jelena Mirkovic: None; Zeina Ghorab: None

Background: Comprehensive immunohistochemical evaluation of tubo-ovarian neoplasms in peritoneal fluids is not universally performed. Yet, when surgical evaluation is not pursued, accurate tumor subtyping/grading in peritoneal fluid specimens is critical given the implications for prospective neoadjuvant therapy. We assessed the degree of immunohistochemical correlation between peritoneal fluid cytology and surgical specimens in an effort to establish the reliability of tumor subtyping and grading in cytology specimens.

Design: Positive pelvic fluid specimens and concurrent/subsequent surgical specimens of borderline or malignant epithelial tubo-ovarian neoplasms were identified. Original cytology and surgical slides were reviewed to confirm the original diagnosis. The number of neoplastic cells and overall tumor cellularity was estimated on cell block slides. Each case pair was immunohistochemically stained with PAX8, WT-1, p53, p16, Napsin-A, ER, PR. For PAX8, WT-1, and Napsin-A, staining intensity (mild, moderate, strong) and percentage of tumor cells staining was recorded. ER/PR expression was assessed as present (any nuclear positivity) or absent. Aberrant p53 expression was defined as strong nuclear staining in >75% of tumor cells (overexpressed) or a complete loss of nuclear staining (null). p16 staining was characterized as overexpressed (diffuse, strong staining in tumor nuclei +/- cytoplasm), patchy (variable, non-block-like), or absent. Kappa values were calculated to assess the correlation between cytology and histology scoring.

Results: A total of 45 case pairs were included (33 high grade serous, 3 clear cell, 1 endometrioid, and 2 low grade serous carcinomas, and 6 serous borderline tumors). The morphology and immunophenotype of all cases was compatible with the final diagnosis. Table 1 depicts the percentage of case pairs with agreement of presence/absence or pattern of expression for each marker and kappa values.

Correlation for PAX8, WT-1, p16, and Napsin-A was perfect. Both instances of p53 and p16 staining disagreement were in cases with ≤ 100 tumor cells. Most case pair disagreements involved PR staining.

Immunohistochemical Marker	*High grade serous carcinoma*	*Clear cell carcinoma*	*Endometrioid adenocarcinoma*	*Low grade serous carcinoma*	*Serous borderline tumour*	*Kappa*
PAX8	100% (33/33)	100% (3/3)	100% (1/1)	2/2 (100%)	100% (6/6)	1.000
WT-1	100% (33/33)	100% (3/3)	100% (1/1)	2/2 (100%)	100% (6/6)	1.000
p53	97% (32/33)	100% (3/3)	100% (1/1)	2/2 (100%)	100% (6/6)	0.966
p16	100% (29/29)	100% (3/3)	100% (1/1)	2/2 (100%)	83% (5/6)	0.912
Napsin-A	100% (33/33)	100% (3/3)	100% (1/1)	2/2 (100%)	100% (6/6)	1.000
Estrogen receptor	97% (32/33)	67% (2/3)	100% (1/1)	2/2 (100%)	100% (4/4)	0.645
Progesterone receptor	61% (19/31)	100% (3/3)	100% (1/1)	2/2(100%)	100% (4/4)	0.424

Conclusions: We have shown a high level of concordance between paired pelvic specimens and surgical specimens, suggesting that high grade serous carcinoma can be distinguished from non-high grade serous carcinomas on pre-surgical cytological preparations. This finding may have implications for early BRCA status testing and choice of therapeutic intervention.

374 Other High Risk HPV Pap Smears and Clinical Outcomes in a Large Academic Center

Melissa Hogan[1], Kim Ely[1], Annie Jack[1]
[1]Vanderbilt University Medical Center, Nashville, TN

Disclosures: Melissa Hogan: None; Kim Ely: None; Annie Jack: None

Background: High risk HPV types 16 and 18 are the most common types of HPV detected in cervical and vaginal Pap smears as a screening test regardless of whether the pap smear has a positive or negative cytology result. In addition, they are subsequently associated with up to 70% of invasive cancer cases. There is scant literature on the natural history on patients with other high-risk (OHR) HPV. In 2013, our institution initiated OHR testing in addition to HPV16 and HPV18 using the Roche Cobas HPV assay. The aim of this project is to evaluate the prevalence of disease progression, as well as, the effect of other possible factors that may contribute to the development of high grade squamous lesions.

Design: A retrospective electronic chart review was performed between Sept. 27,3013 and Nov. 18,2013 (3500 patients) for the first 194 patients with any positivity for OHR (181 positive for OHR only, 11 OHR + 16, 1 OHR + 18 and 1 OHR +16 +18).

Results: The cohort (average age 44, range 28-85) consisted of Caucasian (73.7%), African American (16.8%), American Indian/Alaska Native (1.1%),Asian (2.6%), Unknown (5.3%), Hispanic (0.5%) patients. Average overall follow-up was 1035 days (2.8 years). At initial Pap smear, 8 patients (4.2%) were diagnosed with high grade intraepithelial lesion (HSIL); 5/8 (63%) were confirmed HSIL on subsequent biopsy. 2/5 (40%) were positive for OHR and both were HIV positive. The first patient (age 44, Caucasian) underwent a total of 5 subsequent pap smears which were either diagnosed as ASCUS or HSIL and was positive for OHR when tested two subsequent times with a follow-up period of 5.6 years. The second patient (age 34, unknown race) had 3 subsequent pap smears (benign, ASCUS, benign), which were all negative for HPV and a follow-up period of 3.6 years.

In the 182 patients without HSIL at initial pap smear, 6 patients developed subsequent HSIL lesions; 1/5 (56, Caucasian) were confirmed on subsequent biopsy and positive for only OHR. This patient was OHR positive with an ASCUS pap at the start of the study and the OHR status persisted through her follow-up period of 5.3 years.

Conclusions: While the prevalence of high grade squamous intraepithelial lesions was low in this study, our data suggests that co-infection with HIV and persistence of OHR HPV infection are causative factors in disease progression. Additional prospective studies with larger patient numbers and longer follow up are needed to further define outcomes of the HPV OHR genotype.

375 Insulinoma-associated Protein 1 (INSM1) Immunostaining in Various Types of Neuroendocrine Tumors on Fine-Needle Aspiration Smears

Tieying Hou[1], Qiong Gan[1], Cicily Joseph[1], Xiaoping Sun[1], Yun Gong[1]
[1]The University of Texas MD Anderson Cancer Center, Houston, TX

Disclosures: Tieying Hou: None; Qiong Gan: None; Cicily Joseph: None; Xiaoping Sun: None; Yun Gong: None

Background: Insulinoma-associated protein 1 (INSM1) has been recently identified as an immunohistochemical marker in the nucleus for neuroendocrine and neuroepithelial neoplasms. In the past few years, several published studies have demonstrated that INSM1 is a reliable marker in the diagnosis of neuroendocrine tumors (NETs) in multiple organ systems including the lung, gastrointestinal and

pancreatobiliary tract. Staining in these studies were performed on formalin-fixed paraffin embedded sections of surgical samples and cytology cell blocks. The utility and reliability of INSM1 immunostaining on cytology smears have not been previously studied. In cytology practice, it is not uncommon that fine-needle aspiration (FNA) smear is the only sample type available for immunostaining workup. In such situation, markers showing nuclear staining usually gives a distinct staining pattern and thus the staining result is easy to interpret compared to markers with cytoplasmic or membranous staining. The purpose of this study is to investigate the reliability of INSM1 in various NETs by comparing its performance with CHR and SYN on smears.

Design: A total of 72 FNA cases of NETs (47 of primary and 25 of metastatic) from 68 patients were included in this study; they were lung small cell carcinoma (SmCC) (n=11), Merkel cell carcinoma (n=10), pancreatic NET (n=20), olfactory neuroblastoma (n=7), thymic atypical carcinoid tumor (n=4), medullary thyroid carcinoma (n=10) and lung carcinoid tumor (n=10). Immunostains of INSM1, CHR and SYN were performed on smears of each case. The percentage of positive cells and intensity of staining were recorded.

Results: The immunohistochemical findings were summarized in Table 1. Compared to CHG and SYN, the detection rate of INSM1 is superior in lung SmCC and Merkel cell carcinoma, equivalent in pancreatic NET, olfactory neuroblastoma and thymic atypical carcinoid tumors, and the same as the rate of CHG and slightly lower than the rate of SYN in medullary thyroid carcinoma and lung carcinoid. Notably, the intensity of staining of INSM1 is comparable to that of the other two markers and the background staining is significantly lower in high grade NETs and/or tumor with extensive necrosis.

Table 1: Results of immunostaining on direct smears

	INSM1			**CHG**			**SYN**		
Tumor (n)	Positive cases (%)	% of positive cells	intensity	Positive cases (%)	% of positive cells	intensity	Positive cases (%)	% of positive cells	intensity
Lung SmCC (11)	10 (91)	78	Weak: 1 moderate: 3 strong: 6	8 (73)	59	Weak: 1 Mod:4 strong: 3	9 (82)	59	Weak: 1 Mod:4 strong: 4
Merkel cell carcinoma (10)	9 (90)	76	Weak: 1 Mod: 4 Strong: 4	5 (50)	81	Mod: 1 Strong: 4	8 (80)	67	Weak: 2 Mod: 3 Strong: 3
Pancreatic NET (20)	20 (100)	74	Weak: 3 Moderate: 17	19 (95)	79	Strong: 20	20 (100)	90	Strong: 20
Olfactory Neuroblastoma (7)	5 (71)	64	Weak: 1 Mod: 3 Strong: 1	5 (71)	75	Weak: 1 Mod: 1 Strong: 2	5 (71)	56	Weak: 1 Mod: 4
Thymic atypical carcinoid (4)	4 (100)	28	Weak: 4	4 (100)	83	Strong: 4	3 (75)	83	Mod: 1 Strong: 2
Medullary thyroid carcinoma (10)	8 (80)	76	Weak: 1 Mod: 1 Strong: 6	8 (80)	100	Strong: 8	9 (90)	97	Weak: 1 Mod: 1 Strong: 7
Lung carcinoid (10)	9 (90)	92	Mod: 5 Strong: 4	9 (90)	96	Mod: 1 Strong: 8	10 (100)	74	Weak: 2 Mod: 3 Strong: 5
Total (72)	65 (90)	70		58 (82)	82		64 (89)	75	

Conclusions: INSM1 can be reliably tested in cytology smears to verify neuroendocrine tumors. The overall detection rate of INSM1 is superior or equivalent to that of CHG and SYN. The nonspecific staining for INSM1 is lower, while its intensity is comparable to that of CHG and SYN.

376 Risk of Malignancy (ROM) in the Category of "Atypia of Undetermined Significance" versus "Suspicious for Malignancy" for Serous Cavity Effusions: A Tertiary Cancer Center's Experience

Tieying Hou[1], Gene Landon[1], John Stewart[1], Sinchita Roy-Chowdhuri[1]
[1]The University of Texas MD Anderson Cancer Center, Houston, TX

Disclosures: Tieying Hou: None; Gene Landon: None; John Stewart: None; Sinchita Roy-Chowdhuri: None

Background: The International Academy of Cytology (IAC) and the American Society of Cytopathology (ASC) have recently proposed an International System for Reporting Serous Fluid Cytopathology (ISRSFC) to provide consistent reporting terminology for these specimens. The five proposed diagnostic categories are "non-diagnostic (ND)", "negative for malignancy (NFM)", "atypia of undetermined significance (AUS)", "suspicious for malignancy (SFM)", and "malignant (MAL)". Due to the limited literature on reporting practice for AUS and SFM groups, and their varying diagnostic accuracy and clinical significance, we retrospectively reviewed our institution's cases in these categories to determine their associated risk of malignancy (ROM).

Design: We reviewed serous effusions between January and December 2014 as AUS or SFM. To calculate ROM, malignancy was confirmed by prior and/or subsequent fluid from the same location and/or tissue biopsy from the same location.

Results: Review of all peritoneal, pleural, and pericardial effusions obtained in this 12-month period identified 145 AUS and 98 SFM cases. Patient demographics and clinicopathologic details are presented in Table 1. The AUS category was typically used when the cells in question lacked the requisite quantitative and/or qualitative features for a definitive diagnosis. In most of these cases, the atypical cells were rare and not present on the cell block, precluding immunohistochemical staining (IHC) (n=123; 85%); however, a subset of these cases (n=51; 35%) were favored to be reactive by cytomorphology. The remaining cases (n=22; 15%) had IHC performed, but with noncontributory or inconclusive results. The ROM in the AUS group was 41%.

In the SFM category, the atypical cells were morphologically suspicious but sparse, precluding IHC (n=36; 37%) or yielding inconclusive IHC results (n=24; 25%). The remaining SFM cases included atypical and/or monomorphic lymphoid population (n=24; 25%) and mesothelial proliferations (n=14; 14%), for which flow cytometry and/or IHC support was lacking or inconclusive. The ROM of the SFM category was 64%.

Table 1: Demographics and Specimen Information

	AUS	**SFM**
No. of patients (n)	132	89
• Female (n)	65	46
• Male (n)	67	43
Age, median (range)	61 (4-91) years	61 (17-86) years
Serous Fluid Source		
• Peritoneal (n)	45	28
• Pericardial (n)	8	3
• Pleural (n)	89	66
Total Volume (ml): median (range)	145 600 (1~3000)	98 1000 (2~2000)
Ancillary Studies Workup		
• Cell block available	27 (19%)	(46%)
• IHC/flow cytometry available	22 (15%)	(37%)
• IHC/flow cytometry not performed	123 (85%)	37%)
• IHC/flow cytometry non-contributory	22 (15%)	(25%)
• Atypical lymphoid cells, SFM*	-	24%)
• Atypical mesothelial proliferation**	-	14 (14%)
Malignancy confirmed by previous and/or subsequent fluid	15	25
Malignancy confirmed by biopsy	14	14
No evidence of malignancy	42	22
ROM	41%	64%

*Flow cytometry not performed and/or non-contributory due to possible peripheral blood contamination

**Clinical/radiological correlation was not available for definitive diagnosis

Abbreviations: AUS, atypia of undetermined significance; IHC, immunohistochemical staining; ROM, risk of malignancy; SFM, suspicious for malignancy;

Conclusions: Our results show that the main reason for cases diagnosed as AUS or SFM is a paucity of atypical or suspicious cells that limits the diagnostic work-up. Additionally, for SFM, some cases remained inconclusive despite ancillary testing. The ROM of SFM was significantly higher than that of AUS (p<0.01), thus supporting the validity to continue maintaining these two groups as independent categories.

377 Fine Needle Aspiration of Non-Mammary Metastases to the Axilla: An Institutional Experience

Tom Hu[1], Roseann Wu[2]
[1]Hospital of the University of Pennsylvania, Philadelphia, PA, [2]University of Pennsylvania Health System, Philadelphia, PA

Disclosures: Tom Hu: None; Roseann Wu: None

Background: Secondary, metastatic involvement of the breast and axilla is an infrequent occurrence (approximately 2-3% of all breast malignancies). The majority of these cases are the result of secondary breast involvement by a hematologic malignancy or metastasis from a contralateral mammary malignancy, with the vast minority of metastatic lesions resulting from a true non-mammary primary. Not surprisingly, patients with non-mammary metastases to the breast or axilla have a poor prognosis, with a median survival ranging from 10-12 months. There is a relative paucity of information regarding the frequency with which non-mammary neoplasms metastasize to the axilla.

Design: We searched the pathology database of the Hospital of the University of Pennsylvania for all axillary fine needle aspirations performed between July 1st, 2008 and July 1st, 2018 in order to identified all cases of non-mammary metastases. Clinicopathologic data were then reviewed to determine the primary tumor diagnosis/site.

Results: 1282 fine needle aspirations were performed. A total of 629 malignancies were identified. Not surprisingly, the majority (383/619, 60.9%) of cases were metastases from mammary malignancies. However, we identified 65 (10.3%) hematolymphoid malignancies as well as 181 cases (28.8%) of non-mammary metastases. Of the noted non-mammary metastases, the most frequently identified primary tumor was melanoma (101/189, 53.4%), followed by thoracopulmonary (26/189, 13.8%), non-melanoma cutaneous (13/189, 6.9%), and head and neck (12/189, 6.3%) malignancies. Figures 1 and 2 summarize our findings.

Non-Mammary Neoplasms	No.	Non-Mammary Neoplasms	No.	Hematolymphoid Neoplasms	No.
Melanoma	**101**	**Genitourinary Tract**	**9**	**Mature B-cell Neoplasms**	**50**
Cutaneous	98	Urothelial	5	Follicular Lymphoma	16
Unknown Primary	3	Prostatic Adenocarcinoma	2	Small Lymphocytic Lymphoma	15
		Renal Cell Carcinoma, Papillary	1	Diffuse Large B-cell Lymphoma	12
Thoracopulmonary	**26**	Renal Cell Carcinoma, Clear Cell	1	Mantle Cell Lymphoma	3
Adenocarcinoma	15			Marginal Zone Lymphoma	2
Mucinous Adenocarcinoma	1	**Gynecologic/Müllerian Tract**	**5**	Lymphoplasmacytic Lymphoma	1
Squamous Cell Carcinoma	3	Ovarian High Grade Serous Carcinoma	2	Plasma Cell Myeloma	1
Combined Small Cell Carcinoma w/ Large Cell Component	1	Primary Peritoneal High Grade Serous Carcinoma	1		
Small Cell Lung Carcinoma	3	Endometrial Serous Carcinoma	1	**Mature T-cell Neoplasms**	**7**
Atypical Carcinoid Tumor	1	Endometrial Endometrioid Carcinoma	1	Peripheral T-cell Lymphoma, NOS	2
Non-small Cell Lung Carcinoma, NOS	2			Anaplastic Large Cell Lymphoma, ALK+	1
Malignant Pleural Mesothelioma, Biphasic	1	**Gastrointestinal Tract**	**4**	Anaplastic Large Cell Lymphoma, ALK-	1
		Colonic Adenocarcinoma	1	T-cell Prolymphocytic Leukemia	1
Skin	**13**	Esophageal Adenocarcinoma	1	Mycosis Fungoides	1
Squamous Cell Carcinoma	8	Esophageal Squamous Cell Carcinoma	1	Sézary Syndrome	1
Merkel Cell Carcinoma	2	Gastro-esophageal Junction Adenocarcinoma	1		
Poorly Differentiated Malignant Adnexal Tumor	1			**Acute Leukemia of Ambiguous Lineage**	**1**
Basal Cell Carcinoma	1	**Sarcoma**	**2**	Mixed Phenotype Acute Leukemia (T/myeloid)	1
Malignant Chondroid Syringoma	1	Undifferentiated Spindle Cell Sarcoma	1		
		Undifferentiated Pleomorphic Sarcoma	1	**Hodgkin Lymphoma**	**7**
Head and Neck	**12**			Classic	4
Papillary Thyroid Carcinoma with Tall Cell Features	1	**Unknown**	**8**	Nodular Lymphocyte Predominant	3
Anaplastic Thyroid Carcinoma	1	Undifferentiated Malignant Neoplasm	3		
Nasopharyngeal Undifferentiated Carcinoma	1	Poorly Differentiated Carcinoma	2		
Nasopharyngeal Non-keratinizing Squamous Cell Carcinoma	1	Squamous Cell Carcinoma	1		
Sinonasal Undifferentiated Carcinoma	1	Carcinoma with Neuroendocrine Differentiation	1		
Oropharyngeal HPV-unrelated Squamous Cell Carcinoma	2	Small Cell Carcinoma	1		
Oral Cavity Conventional Squamous Cell Carcinoma	3				
Parotid Gland High Grade Acinic Cell Carcinoma	1				
Parotid Gland Primary Squamous Cell Carcinoma	1				

Figure 1 - 377

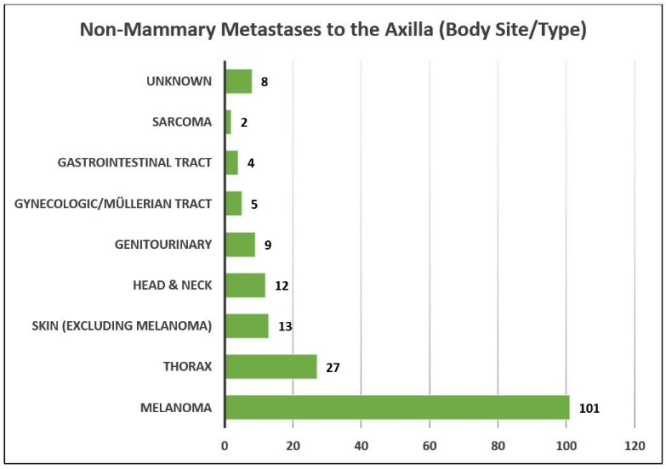


Figure 2 - 377

Non-Mammary Neoplasms	No.	Non-Mammary Neoplasms	No.	Hematolymphoid Neoplasms	No.
Melanoma	**101**	**Genitourinary Tract**	**9**	**Mature B-cell Neoplasms**	**50**
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Malignant Pleural Mesothelioma, Biphasic	1	**Gastrointestinal Tract**	**4**	Anaplastic Large Cell Lymphoma, ALK-	1
		Colonic Adenocarcinoma	1	T-cell Prolymphocytic Leukemia	1
Skin	**13**	Esophageal Adenocarcinoma	1	Mycosis Fungoides	1
Squamous Cell Carcinoma	8	Esophageal Squamous Cell Carcinoma	1	Sézary Syndrome	1
Merkel Cell Carcinoma	2	Gastro-esophageal Junction Adenocarcinoma	1		
Poorly Differentiated Malignant Adnexal Tumor	1			**Acute Leukemia of Ambiguous Lineage**	**1**
Basal Cell Carcinoma	1	**Sarcoma**	**2**	Mixed Phenotype Acute Leukemia (T/myeloid)	1
Malignant Chondroid Syringoma	1	Undifferentiated Spindle Cell Sarcoma	1		
		Undifferentiated Pleomorphic Sarcoma	1	**Hodgkin Lymphoma**	**7**
Head and Neck	**12**			Classic	4
Papillary Thyroid Carcinoma with Tall Cell Features	1	**Unknown**	**8**	Nodular Lymphocyte Predominant	3
Anaplastic Thyroid Carcinoma	1	Undifferentiated Malignant Neoplasm	3		
Nasopharyngeal Undifferentiated Carcinoma	1	Poorly Differentiated Carcinoma	2		
Nasopharyngeal Non-keratinizing Squamous Cell Carcinoma	1	Squamous Cell Carcinoma	1		
Sinonasal Undifferentiated Carcinoma	1	Carcinoma with Neuroendocrine Differentiation	1		
Oropharyngeal HPV-unrelated Squamous Cell Carcinoma	2	Small Cell Carcinoma	1		
Oral Cavity Conventional Squamous Cell Carcinoma	3				
Parotid Gland High Grade Acinic Cell Carcinoma	1				
Parotid Gland Primary Squamous Cell Carcinoma	1				

Conclusions: In our series, the most common non-mammary non-hematolymphoid malignancy metastatic to the axilla was melanoma (53.4%). In addition to melanoma, however, we discovered a wide variety of metastatic malignancies from various other sites, most commonly the lung (13.8%), skin (6.9%), and the head and neck (6.3%). Metastases to the axilla with non-mammary primary sites are rare occurrences associated with poor patient outcomes, and thus must be distinguished from metastases to the axilla from a primary mammary malignancy. Thankfully, a clinical history of a known non-mammary primary was available in the vast majority of our cases. Fine needle aspiration is a non-invasive and cost-effective way to assess involvement of axillary sites by mammary, non-mammary, and hematolymphoid malignancies.

378 Cytopathologist Assessment of Cell Block for Panel Molecular Testing in Pancreatic Carcinoma Specimens Improves Adequacy Rates

Yuan Huang[1], Yingchun Wang[2], Shobha Parajuli[1], Paul Lee[2]
[1]University of Cincinnati Medical Center, Cincinnati, OH, [2]University of Cincinnati, Cincinnati, OH

Disclosures: Yuan Huang: None; Yingchun Wang: None; Shobha Parajuli: None; Paul Lee: None

Background: In the era of personalized medicine, requests for molecular testing on endoscopic ultrasound fine needle aspirations (EUS-FNA) pancreas specimens are increasing. At our institution, cell blocks are prepared on all pancreatic EUS-FNA specimens. When a limited molecular panel testing is requested, we utilize the cell block as the basis for the required genetic material. However, many times the cell blocks are sent without assessment by a cytopathologist, resulting in many samples being deemed quantity not sufficient (QNS). The overall aim of this study is to assess our molecular QNS rates and understand causes for inadequacy rates.

Design: 25 cytology cases of pancreatic carcinomas between January 2015 and August 2019 with concurrent clinically validated next-generation sequencing (NGS) panel testing were identified. The cell block slides were reviewed independently in a blinded-fashion by three board-certified cytopathologists. The overall adequacy and cellularity were assessed in a three-tiered fashion. An adequate sample is defined by at least two cytopathologist's agreement or average cellularity higher than 2. The interrater agreement was measured by Fleiss's statistics. Viable tumor percentage (V%) was evaluated by looking at tumor cells in the cellular component. Global tumor percentage (G%) was assessed for both cellular and non-cellular components. The mutational allele frequency (MAF) from NGS data was compared to the assessed G% and V% by Pearson correlation.

Results: The overall QNS rate for molecular panel testing in pancreatic cytology cases was 12/25 (48%). Upon cytopathologist evaluation based on overall adequacy, 8/12 (67%) of the QNS cases and 12/13 (92%) of the adequate cases were correctly predicted, with an accuracy of 80% and sensitivity of 92%. When using the cellularity criteria, 6/12 (50%) of the QNS cases were deemed inadequate, while 9/13 (69%) of the adequate cases were deemed adequate, resulting in an accuracy of 60% and sensitivity of 69% (Figure 1). Both assessing criteria showed moderate interrater agreement (Figure 2). Neither V% or G% was correlated with expected MAF (R = -0.15 and 0.18, respectively). Stroma (4/7, 57%) was the most common non-tumor cellular component, with blood and necrosis (5/7, 71%) being the most common non-cellular component identified.

Figure 1 - 378

Criteria	Adequacy for testing	Tested Cases	QNS Cases	Accuracy
Overall Adequacy	Adequate	12	4	80%
	Inadequate	1	8	
		Sensitivity (92%)	Specificity (67%)	
Cellularity	Adequate	9	6	60%
	Inadequate	4	6	
		Sensitivity (69%)	Specificity (50%)	

Figure 2 - 378

Interrater Agreement in Assessing Pancreatic Cytology Specimen Adequacy *

Criteria / Category	Overall Adequacy	Cellularity
Overall kappa [95%CI]	0.60 [0.44, 0.76]	0.46 [0.29, 0.62]
Tier 1: kappa	0.71 ($p < 0.01$)	0.40 ($p < 0.01$)
Tier 2: kappa	0.37 ($p < 0.01$)	0.18 ($p > 0.05$)
Tier 3: kappa	0.71 ($p < 0.01$)	0.58 ($p < 0.01$)

* The level of interrater agreement was graded based on the Landis and Koch scale: <0 as poor, 0.01 – 0.20 as slight, 0.21 – 0.40 as fair, 0.41 – 0.60 as moderate, 0.61 – 0.80 as substantial, and > 0.80 as nearly perfect.

Conclusions: Cytopathologist assessing adequacy for NGS panel molecular testing can improve adequacy rates with increased accuracy and sensitivity in pancreas cytology cases.

379 The Utility of UroVysion® as an Intravesical Recurrence Predictor after Radical Nephroureterectomy for Urothelial Carcinoma of the Upper Urinary Tract

Hidehiro Iwata[1], Naoto Sassa[2], Mashashi Kato[2], Ryohei Hattori[3], Taishi Takahara[4], Momokazu Goto[5], Toyonori Tsuzuki[6]
[1]Japanese Red Cross Nagoya Daini Hospital, Nagoya-shi, Aichi, Japan, [2]Nagoya University Hospital, Nagoya, Japan, [3]Japanese Red Cross Nagoya Daiichi Hospital, Nagoya, Japan, [4]Aichi Medical University Hospital, Nagakute, Japan, [5]Nagoya University Hospital, Nagoya, Aichi, Japan, [6]Aichi Medical University Hospital, Nagakute, Aichi, Japan

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Background: Intravesical recurrence (IVR) after radical nephroureterectomy (RNU) for urothelial carcinoma of the upper urinary tract (UCUUT) is a major recurrence event with a frequency of 22.47%. One of the major mechanisms is the implantation of cancer cells from UCUUT. Therefore, the detection of cancer cells in the bladder after RNU can be a predictor of IVR. This study aimed to examine the utility of UroVysion® as a predictor of IVR using bladder urine after RNU for UCUUT.

Design: We prospectively enrolled 64 patients who had undergone RNU for high-grade UCUUT at the Nagoya University Hospital and Nagoya Daiichi Red Cross Hospital between October 2013 and April 2017. Of these, we selected 53 patients who could provide bladder urine samples just after RNU (0POD) and 5 days after RNU (5POD). Results from UroVysion® were considered positive if (1) 10% or more of cells had polysomy of three chromosomes, (2) 10% or more of cells had polysomy of two chromosomes and deletion of 9p21, or (3) 30% or more of cells had a deletion of 9p21. At least 25 cells were analyzed in each enrolled case. Moreover, we performed urine cytology according to the Paris System using the same samples. Cases with atypical urothelial cells were regarded as positive. The IVR rate was analyzed by the log-rank test. Sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) of cytology and UroVysion® results were analyzed by the Fisher exact test. A value of $p < 0.05$ was considered statistically significant.

Results: IVR of the tumor was confirmed in 26 patients by transurethral resection of the bladder tumor. This included 25 0POD- and 23 5POD-positive patients and 22 patients who showed 0POD or 5POD positivity. The IVR rate was significantly different between the group positive for 0POD or 5POD and the group negative for both according to UroVysion® (p=0.004) and cytology (p=0.013). The sensitivity, specificity, PPV, and NPV values for UroVysion® were 95.5% (21/22), 35.5% (11/31), 51.2% (21/41), and 91.7% (11/12), respectively and for cytology, the values were 68.2% (15/22), 54.8% (17/31), 51.7% (15/29), and 70.8% (17/24), respectively (Table 1). UroVysion® demonstrated significantly higher sensitivity than cytology (p=0.019). The IVR rate between each 0POD and 5POD was not significantly different according to UroVysion® and

Table 1. Comparison of UroVysion® and cytology for both 0POD and 5POD cases

	UroVysion®	Cytology	p-value
Sensitivity	95.5% (21/22)	68.2% (15/22)	0.019
Specificity	35.5% (11/31)	54.8% (17/31)	0.126
PPV	51.2% (21/41)	51.7% (15/29)	0.966
NPV	91.7% (11/12)	70.8% (17/24)	0.156

Conclusions: Multiple urine tests with UroVysion® after RNU could be a useful predictor of IVR. Although cytology tests were useful, their utility was inferior to UroVysion®.

380 Are Age and Sex Predictors of Malignancy in Cytologically Indeterminate Thyroid Nodules?

Pari Jafari[1], Daniel Johnson[2], Allison Cavallo[3], Imran Uraizee[4], Tanaz Vaghaiwalla[5], Peter Angelos[4], Tatjana Antic[4], Nicole Cipriani[4]
[1]University of Chicago Pritzker School of Medicine, Chicago, IL, [2]Northwestern University Feinberg School of Medicine, Chicago, IL, [3]Northwestern Memorial Hospital System, McHenry, IL, [4]The University of Chicago, Chicago, IL, [5]University of Chicago Medical Center, Chicago, IL

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Background: Within the Bethesda System for Reporting Thyroid Cytology (BSRTC), indeterminate nodules (categories III, IV, and sometimes V) pose a diagnostic challenge. While age and sex correlate with thyroid nodule rates of malignancy (ROM) on final resection (namely, males and younger patients are at greatest risk of malignancy), their predictive value at the cytopathologic level, particularly among indeterminate nodules, remains to be fully elucidated.

Design: We identified 618 thyroid nodules resected between 2011 and mid-2019 at a tertiary care center that were pre-operatively diagnosed by in-house cytopathologists as Bethesda categories III-V. Bethesda III was subdivided based on nuclear atypia (atypia of undetermined significance, AUS) and microfollicular architecture (follicular lesion of undetermined significance, FLUS). Bethesda categories were then correlated with final histologic diagnosis for each nodule. Nodule location, size, and imaging/clinical characteristics were paired with gross description at resection in order to ensure accuracy of correlation. Microcarcinomas identified outside the dominant nodule(s) were not considered malignant for the purposes of this study. For each Bethesda category, patients were stratified by sex and

age (pediatric, 0-21; adult, 22-74; elderly, 75+) and by age subgroups for adults (22-44, 45-54, 55-74). The Kruskal-Wallis test was used to compare ROM across age groups and subgroups for each Bethesda category. Odds ratios were used to compare ROM between males and females of all ages within each Bethesda category.

Results: Within Bethesda categories III, IV, and V, the Kruskal-Wallis test revealed no significant difference in ROM among age groups and subgroups (p>0.4). For III-AUS and III-FLUS, only comparison of the adult subgroups was possible; again, there were no significant differences in ROM (p>0.1).

For each Bethesda category, including III-AUS and III-FLUS, comparison of ROM by sex revealed no statistically significant differences (p>0.1).

Percent Malignant by Bethesda Category vs. Age

		Age (years)						
Bethesda Category		**0-21**	**22-74**	**22-74**			**75+**	**All ages**
		n=24	*n=561*	**22-44**	**45-54**	**55-74**	*n=33*	*n=618*
				n=159	*n=127*	*n=275*		
III-all		42.9	16.8	19.5	8.3	18.7	5.9	16.8
	III-AUS	33.3	35	47.8	13.6	38.2	16.7	33.9
	III-FLUS	50	7.3	8.5	5.3	7.4	0	7.7
IV		44.4	24.2	18.4	27.3	25.6	36.4	26
V		62.5	80.8	84.6	79.4	78.7	80	79.7

Conclusions: While sample size was somewhat limited for pediatric (≤21-year-old) and elderly (≥75-year-old) populations, there were no significant differences in ROM across adult age subgroups. Additionally, comparison of males and females revealed no significant difference in ROM within a Bethesda category. These findings suggest that across age groups and between sexes, BSRTC is an equally efficacious predictor of malignancy upon resection.

381 Performance Characteristics of Renal Fine Needle Aspiration in a Tertiary Care Center over a 20-Year Period

Ayse Irem Kilic[1], Kubra Katipoglu[2], Eva Wojcik[1], Stefan Pambuccian[1], Güliz Barkan[3]
[1]Loyola University Medical Center, Maywood, IL, [2]Hacettepe University, Ankara Türkiye, Ankara, Turkey, [3]Loyola University Healthcare System, Maywood, IL

Disclosures: Ayse Irem Kilic: None; Kubra Katipoglu: None; Eva Wojcik: None; Stefan Pambuccian: None; Güliz Barkan: None

Background: There have been increasing indications for renal fine-needle aspiration (FNA) with or without concurrent core needle biopsy (CNB) based on the clinical guidelines for management of renal lesions. In this study we aimed to evaluate the diagnostic performance of renal FNA cases with or without CNB in our institution.

Design: The electronic medical records of our institution were searched for all renal FNA specimens from 01/01/2000 through 06/30/2019. The patient's age, sex, radiologic image, diagnosis, presence of CNB, and follow-up information (surgery/clinical status) were tabulated. The diagnoses were classified into 5 categories: non-diagnostic, negative, neoplastic, atypical, suspicious for malignancy and malignant. The radiologic imaging were categorized as cystic, mixed (solid and cystic), and solid.

Results: A total of 202 cases of FNA with 55 (27.2%) concurrent CNB and 72 (35.6%) follow-up nephrectomy specimens and surgical biopsies were identified. The 202 patients, 104 (51.5%) were male, 98 (48.5%) were female with an age range of 6 to 88 years (median=63). There were only 3 pediatric cases (<18years) (1.5 %), diagnosed as angiomyolipoma (2) and benign cyst (1). The combined diagnostic rate of FNA and concurrent CNB was significantly higher than FNA alone (p<0.05) (Table-1). Considering nephrectomy diagnosis as the gold standard, the sensitivity of FNA was 70% while the combined sensitivity of FNA and CNB was 94% and both of the procedures have 100% specificity with 76% and 96% diagnostic accuracy respectively. The nondiagnostic rate was 21/112 (18.75%) and 2/29 (6.9%) on solid and cystic lesions respectively. There was no difference between the diagnostic rates for solid and cystic lesions (p>0.05). The malignant diagnoses rate on FNA was 94/202 (46.5%), and mostly included renal cell carcinoma and its variants (Table-2). The presence of concurrent CNB was decreased by the age of the patients (p<0.05) while both FNA and CNB was increased by the year since 2000 (p<0.05) (Figure).

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Table-1 Diagnosis of Renal Lesions by FNA alone, CNB alone and Combined FNA and CNB			
Diagnosis	FNA*	CNB**	FNA plus CNB
Nondiagnostic	29 (14.4%)	4 (7.3%)	1 (1.8%)
Negative	50 (24.7%)	9 (16.4%)	9 (16.4%)
Neoplasm	18 (8.9%)	5 (9.1%)	4 (7.3%)
Atypical	4 (2.0%)	0 (0%)	0 (0%)
Suspicious for malignancy	7 (3.5%)	2 (3.6%)	1 (1.8%)
Malignant	94 (46.5%)	35 (63.6%)	40 (72.7%)
Total	202	55	55
Diagnostic rate	85.6%	92.7%	98.2%
P<0.008 difference between diagnostic rate of FNA vs combined FNA and CNB *FNA: Fine-needle aspiration **CNB: Core-needle biopsy			

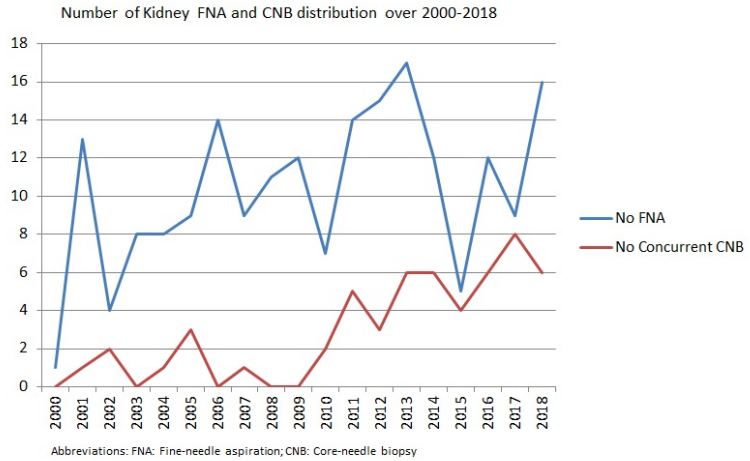

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Table-2 Correlation of Kidney FNA Diagnosis with Nephrectomy Specimens		
FNA Diagnosis	No. of Follow-up Nephrectomies (%)	Nephrectomy diagnosis
Nondiagnostic (29 cases)	12 (16.7%)	RCC, clear cell (9 cases) RCC, chromophobe (2 cases) RCC, papillary (1 case)
Negative (50 cases)	16 (22.2%)	Benign cyst/pseudocyst/abscess (11 cases) RCC, clear cell (3 cases) RCC, papillary (1 case) RCC, unclassified (1 case)
Neoplasm (18 cases)	6 (8.3%)	RCC, clear cell (1 case) RCC, chromophobe (1 case) Oncocytoma (2 cases) Angiomyolipoma (2 cases)
Atypical (4 cases)	1 (1.4%)	RCC, clear cell (1 case)
Suspicious for malignancy (7 cases)	2 (2.8%)	RCC ,clear cell (2 cases)
Malignant (94 cases)	35 (48.6%)	RCC, clear cell (9 cases) RCC, papillary (11 cases) RCC , chromophobe (3 cases) RCC, eosinophilic (2 cases) RCC, translocation type (1 case) RCC, sarcomatoid (2 cases) RCC, unclassified (1 case) RCC, poorly differentiated (1 case) Wilms tumor (1 case) UCC, high grade (2 cases) DLBCL (1 case) Metastatic SCC (1 case)
Total	72	
Abbreviations: FNA: Fine-needle aspiration; RCC: Renal cell carcinoma; UCC: Urothelial cell carcinoma; DLBCL: Diffuse large B-cell lymphoma; SCC: Squamous cell carcinoma		

Conclusions: The study showed the combined diagnostic rate of FNA and CB was better than FNA alone; however the specificity of FNA alone was 100%. The relatively lower sensitivity of FNA alone is due to the availability of rapid onsite evaluation on all renal FNA cases, and for "inadequate" specimens radiologists mostly prefer to perform a concurrent CNB. Also, notably the radiologic characteristics of the lesion (solid/cystic etc.) did not affect the diagnostic rate of FNA.

382 Should We Review Fine-Needle Aspiration and Core Biopsies Procured during the Same Procedure Together or Separately?

Ayse Irem Kilic[1], Aaron Muhlbauer[2], Swati Mehrotra[1], Eva Wojcik[1], Güliz Barkan[3], Stefan Pambuccian[1]
[1]Loyola University Medical Center, Maywood, IL, [2]Loyola University Medical Center, Chicago, IL, [3]Loyola University Healthcare System, Maywood, IL

Disclosures: Ayse Irem Kilic: None; Aaron Muhlbauer: None; Swati Mehrotra: None; Eva Wojcik: None; Güliz Barkan: None; Stefan Pambuccian: None

Background: Fine needle aspiration (FNA) and core biopsies (CoBx) are increasingly used in daily practice to diagnose mass lesions or imaging abnormalities of various organs. Increasingly these procedures are used together, with or without rapid on-site examination (ROSE) of the aspirate. The workflow of different institutions differs in regard to which service (and pathologist) the FNA and CoBx are assigned, and therefore if they are evaluated together by the same pathologist or separately by different pathologists. In our institution, we have implemented a change in 2015, where FNA+CoBx have been assigned to the same pathologist and all cytopathologists also sign out the surgical pathology cases. The aim of this study is to investigate the performance criteria of FNA and CoBx when signed out by the same pathologist or by separate pathologists.

Design: We reviewed our experience with concomitant FNA and CoBx that were signed out by the same pathologist or by different pathologists from 2010 to 2019. Data regarding the site sign-out pathologist, biopsies, stains performed on FNA cell block sections and CoBx; and FNA and CoBx diagnoses were tabulated. Correlation between FNA and CoBx, rates of unsatisfactory, negative, atypical, suspicious and positive diagnoses were calculated for both FNA and CoBx and were compared with the Fisher exact test.

Results: 353 combined FNA+CoBx (105 lung, 176 liver, 19 pancreatic, 29 renal, 8 lymph nodes and 16 miscellaneous sites) were performed in the 10 year period of this study; 258 were signed out by the same pathologist, while 95 were signed out by different pathologists. The diagnostic rates are shown in Table 1. The “positive” diagnosis rate of the combination of FNA and CoBx was superior to either FNA alone or CoBx alone. We also found a significant difference in cytology-histology correlation (i.e. same diagnosis made on both FNA and CoBx) as well as "positive diagnosis rate" when comparing cases signed out by the same pathologist versus two different pathologists.

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Table-1: Diagnostic rates of FNA and CoBx by Sign-out Pathologists

	Non-Diagnostic		Positive for Malignancy	
	FNA	CoBx	FNA	CoBx
Same Pathologist	18 (7%)	8 (3.1%)	185 (71.7%)	204 (79.1%)
Different Pathologists	19 (20%)	6 (6.3%)	43 (45.3%)	53 (55.8%)
P value	*<0.0001*			

	Same Diagnosis	CoBx with worst diagnosis >FNA	FNA with worst diagnosis>CoBx	Total
Same Pathologist	210 (81.4%)	35 (13.6%)	13 (5%)	258
Different Pathologists	63 (66.3%)	25 (26.3%)	7 (7.4%)	95
P value	*0.004*			

**P values refer to comparisons between cases signed out by the same pathologist, compared to those signed out by 2 different pathologists.*
Abbreviations: FNA: Fine-needle aspiration; CoBx: Core Biopsy

Conclusions: We found that sign-out of FNA+CoBx by the same pathologist resulted in better agreement between the two and a higher diagnostic rate of the combination of FNA+CoBx. These results may not be generalizable to the other practice settings.

383 Standardizing a Volume Benchmark for Cerebrospinal Fluids (CSF) for Optimal Diagnostic Accuracy

David Kim[1], Samuel Gilbert[2], Susan Alperstein[3], Momin Siddiqui[1]
[1]New York-Presbyterian/Weill Cornell Medical Center, New York, NY, [2]New York-Presbyterian/Weill Cornell Medicine, New York, NY, [3]Weill Cornell Medicine, Fishkill, NY

Disclosures: David Kim: None; Samuel Gilbert: None; Susan Alperstein: None; Momin Siddiqui: None

Background: Cytomorphologic examination of CSF is a mainstay in the evaluation of leptomeningeal involvement by malignancies. However, there is no set standard for an optimal volume of CSF that is needed to achieve an accurate diagnosis. In this study, we investigated the volumes of CSF sent for detection of leptomeningeal involvement by malignant cells in order to determine if there is a difference in satisfactory and unsatisfactory samples.

Design: CSF specimens from 2014 to 2018 sent for cytomorphologic analysis were retrospectively identified in our laboratory information system. 4,183 CSF samples with recorded volumes were identified. These cases were then categorized as; unsatisfactory (n = 12), negative (n = 3,818), atypical (n = 242), and positive/suspicious (n = 152). Additionally, cases were grouped into unsatisfactory (n = 501) or satisfactory (n = 3417) categories. Positive/suspicious were further analyzed based upon the malignancy detected as carcinoma (n = 29), leukemia (n = 70), or lymphoma (n = 53). Average volumes were calculated for each category and compared using a t-test to identify significance.

Results: The mean volume for satisfactory CSF samples is 7.3 mL (CI 95%, 7.2, 7.5) compared to 6.9 mL (CI 95%, 4.6, 9.2) for unsatisfactory samples and 6.7 mL (CI 95%, 6.4, 7.0) for acellular samples (Figure 1). A t-test revealed a statistically significant difference in volume between the acellular and satisfactory datasets. When acellular and unsatisfactory specimens were grouped it produced a statistically different mean of 6.69 (CI 95%, 6.39, 7.01) from the satisfactory samples. For positive/suspicious cases, mean volumes of 10.3 mL for carcinomas (CI 95%, 7.8, 12.8), 6.4 mL for leukemias (CI 95%, 5.6, 7.1), and 8.4 mL for lymphomas (CI 95%, 7.1, 9.6) were calculated with a statistically significant difference between the carcinoma and leukemia groups as well as the leukemia and lymphoma groups (Figure 2).

Figure 1 - 383

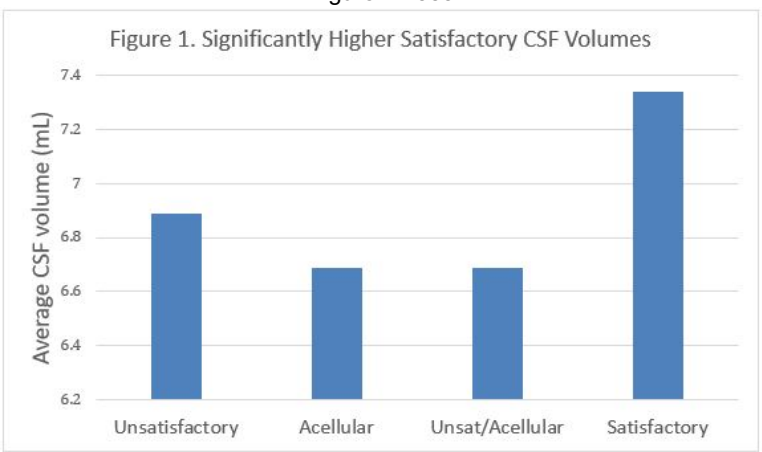
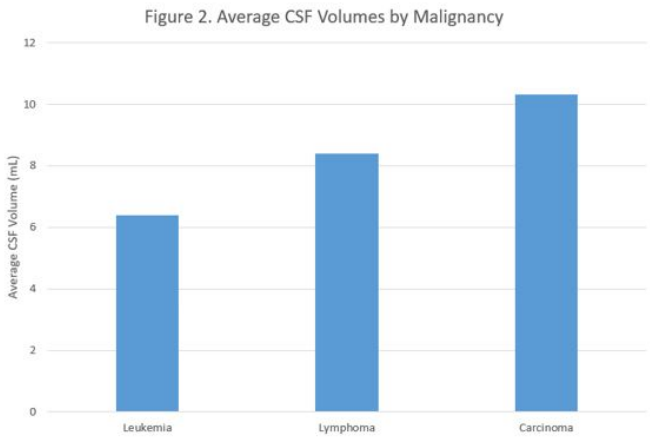


Figure 2 - 383



Conclusions: In our study, our results suggest that higher volumes produce better results for analysis. Based on our data, we recommend 7.3 mL as an optimal CSF volume for cytologic work-up of malignancies. Additionally, depending on the type of malignancy suspected differing minimum volumes yield better specimens. A minimum CSF collection volume(s) of 10.3 ml, 6.4 ml and 8.4 ml for clinical work up for metastatic carcinoma, leukemia's and lymphomas, respectively can additionally be followed as a guideline if clinically warranted.

384 Use of Immunohistochemistry to Distinguish Normal Gastric and Duodenal Mucosa from Intraductal Papillary Mucinous Neoplasms

Patricia Kim[1], Fan Lin[1], Jianhui Shi[1], Angela Bitting[1], Tina Brosioius[1], Haiyan Liu[1]
[1]Geisinger Medical Center, Danville, PA

Disclosures: Patricia Kim: None; Fan Lin: None; Jianhui Shi: None; Angela Bitting: None; Tina Brosioius: None; Haiyan Liu: None

Background: Fine needle aspiration (FNA) is a widely used method of assessing pancreatic lesions. Morphologic distinguishing between gastric and/or duodenum mucosal 'contaminants' from intraductal papillary mucinous neoplasm (IPMN) without high-grade dysplasia in FNA samples is difficult. Only few published reports evaluate the use of immunohistochemistry (IHC) to make this distinction. In this study, we evaluated CK7, synaptophysin (SYN), MUC2 and MUC5AC expression in IPMNs, normal duodenum and stomach, to explore the diagnostic utility of IHC.

Design: IHC evaluation of CK7 (OV-TL 12/30), SYN (MRQ-40), MUC2 (MRQ-18), and MUC 5AC (MRQ-19) from Cell Marque was performed on tissue sections of 21 IPMNs, normal duodenum and stomach from Whipple resections. The staining intensity was recorded as strong (S) or weak (W); distribution recorded as negative (<5% cell staining), 1+ (5-25%), 2+ (25-50%), 3+ (50-75%) and 4+ (>75%).

Results: The staining results are summarized in Tables 1. CK7 is strongly positive in 100% of IPMNs, with the majority (14/21) being diffuse (Fig.1); but negative in normal duodenal mucosa and focal weakly positive in gastric superficial mucosa. SYN decorated neuroendocrine cells in normal gastric and duodenum mucosa in a distinct single cell pattern (Fig.2), only focal weakly in rare IPMN cases.

Table 1. Summary of IHC Results on 21 Cases of IPMNs, normal duodenum and stomach

Antibody/Tissue		**IPMN (n=21)**	**Duodenum (n=21)**	**Stomach (n=20)**
CK7	**1+ w/s (N)**	0/3	0	5/0
	2+ w/s (N)	1/3	0	5/0
	3+ w/s (N)	0/2	0	2/0
	4+ w/s (N)	0/12	0	0
	Pos.%	**100%**	0%	60%
	Pattern	Diffuse	Rare single cells in crypt	Superficial, W and focal; rare single cells in crypt
SYN	**1+ w/s (N)**	2/0	0/21	0/20
	2+ w/s (N)	2/1	0	0
	3+ w/s (N)	0	0	0
	4+ w/s (N)	0	0	0
	Pos.%	24%	100%	100%
	Pattern	Focal, weak	**Single NE cells** in crypt	**Single NE cells** in crypt
MUC2	**1+ w/s (N)**	0/2	0/21	0
	2+ w/s (N)	0	0	0
	3+ w/s (N)	1/2	0	0
	4+ w/s (N)	0/7	0	0
	Pos.%	57%	100%	**0%**
	Pattern	Diffuse	**Goblet cells in villi**	Negative
MUC5AC	**1+ w/s (N)**	0/1	0	0
	2+ w/s (N)	0	0	0
	3+ w/s (N)	0/2	0	0
	4+ w/s (N)	2/16	0	**0/20**
	Pos.%	100%	**0%**	100%
	Pattern	Diffuse	Rare cells strong	Superficial, diffuse strong

Figure 1 - 384

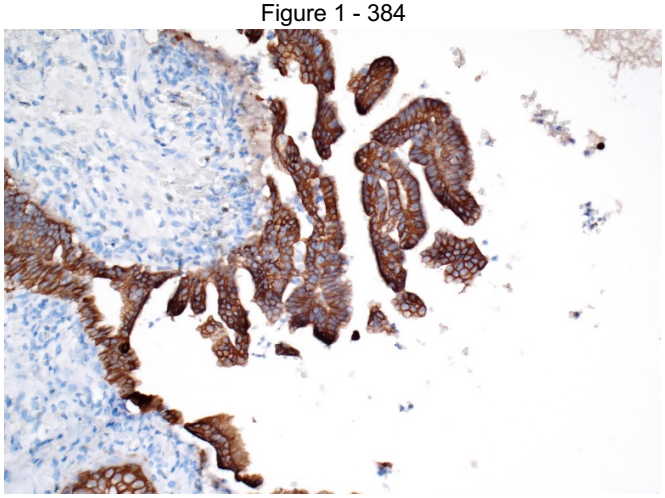
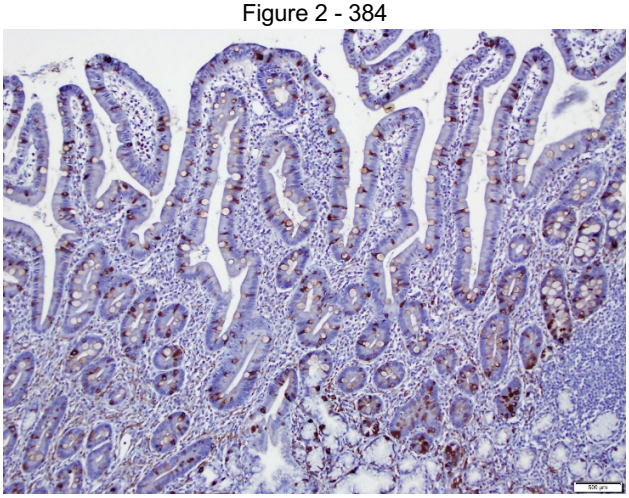


Figure 2 - 384



Conclusions: Our data suggest: 1. Diffuse CK7+ is characteristic of IPMN; 2. distinct strong single cell staining pattern for SYN favors duodenum and/or gastric mucosal contaminants; 3. diffuse MUC2 expression excludes mucosal contaminants from duodenum and/or stomach. The phenotype of CK7+/MUC2+ (diffuse)/SYN- highly suggests IPMN of pancreas. Additional studies on FNA samples are necessary to validate the findings.

385 Validation of Estrogen Receptor Immunohistochemistry on Cell Blocks from Breast Cancer Specimens in Tanzania

Asteria Kimambo[1], Edda Vuhahula[1], Amos Mwakigonja[2], Katherine Van Loon[3], Dianna Ng[4]
[1]Muhimbili University of Health and Allied Sciences, Dar es Salaam, Tanzania, [2]MUHAS, Dar es Salaam, Tanzania, [3]University of California San Francisco, San Francisco, CA, [4]University of California San Francisco Medical Center, San Francisco, CA

Disclosures: Asteria Kimambo: None; Edda Vuhahula: None; Amos Mwakigonja: None; Katherine Van Loon: None; Dianna Ng: *Primary Investigator*, Cepheid, Inc.

Background: Breast cancer is the second leading cause of cancer-related mortality among females in Tanzania. Assessment of biomarkers for breast cancer is critical for therapeutic planning and determining prognosis. In Tanzania, hormone receptor and HER2 immunohistochemistry (IHC) are currently performed on surgical specimens only, which are subject to suboptimal tissue fixation. Performing IHC on cell blocks offer advantages, including immediate fixation and avoidance of unnecessary surgeries in patients with metastatic disease. Therefore, we aimed to evaluate the feasibility of assessing breast cancer biomarkers on fine needle aspiration material by performing ER IHC on cell blocks for the first time in Tanzania.

Design: Patients with breast masses were identified prospectively from the FNAB clinic at a large national referral hospital in Tanzania. Patients with masses that were suspicious for malignancy or malignant by rapid on site evaluation (ROSE) were recruited. FNAB material was rinsed into 10% neutral buffered formalin and cell blocks were prepared using agarose. ER IHC on cell blocks was compared with ER testing on corresponding surgical specimens.

Results: A total of 87 female patients provided informed consent, with a median age of 50 years (range: 25-84). Among these women, 52 (60%) presented with lesions that were >5cm, and 55 (63%) had palpable axillary lymph nodes. All cases received a diagnosis of adenocarcinoma by ROSE and on final cytologic review. There were 63 cases (72%) with corresponding surgical specimens and ER IHC, of which 57 (90%) were confirmed as invasive ductal carcinoma. There were 62 pairs with available cell blocks and surgical specimens with sufficient tumor cellularity. Overall ER IHC concordance was 90%. Positive concordance was 88% (0.8 Kappa, p = 0.7). Sensitivity and specificity were 88% (95% CI: 72-97%) and 93% (95% CI: 77-99%), respectively.

Conclusions: ER IHC on cell blocks showed excellent concordance with the corresponding surgical specimen. ER IHC on cell blocks is feasible and can be implemented in resource-constrained facilities using the developed protocol. The method allows women to receive ER status results using tissue collected at the time of initial FNAB and diagnosis of breast cancer, with potential to improve timely access to hormonal therapy and/or to avoid unnecessary surgeries. Moreover, the ability to perform IHC on cell blocks in Tanzania significantly builds diagnostic capacity and can be applied to a broad range of tumors.

386 ThyroSeq Next-Generation Sequencing in Cytologically Indeterminate Thyroid Nodules – Experience of a Single Institution

Michael Lai[1], Arianne Foster[1], William Klump[2], Lisa Reid[2], Farah Morgan[2], Shuyue Ren[2]
[1]Cooper Medical School of Rowan University, Camden, NJ, [2]Cooper University Hospital, Camden, NJ

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Background: Next-generation sequencing (NGS) has allowed for rapid detection of detailed genetic alterations. With the accelerated discovery of thyroid cancer markers, NGS has proved to be an increasingly common adjunctive test for thyroid nodules diagnosed as indeterminate by cytopathology. ThyroSeq version 3 is a DNA and RNA-based NGS assay that analyzes 112 genes for a variety of genetic alterations and uses a Genomic Classifier to separate malignant from benign lesions. The purpose of this study is to assess the ThyroSeq NGS performance at a single academic tertiary medical center.

Design: Retrospective analysis was performed on thyroid nodules diagnosed by Bethesda System for reporting thyroid cytopathology as category III (Atypia of undetermined significance [AUS]/Follicular Lesion of Undetermined Significance [FLUS]) and category IV (suspicious for Follicular Neoplasm [SFN]/Follicular Neoplasm [FN]) evaluated with ThyroSeq NGS between March 2018 and June 2019 at an Endocrinology practice in a single academic medical center. Calculations for overall positive predictive value (PPV), negative predictive value (NPV), specificity, sensitivity, false positive, and false negative calculations were performed.

Results: 266 thyroid nodules were evaluated and 32 nodules were diagnosed as AUS/FLUS and SFN/FN by Bethesda System for reporting thyroid cytopathology. Out of 29 nodules with ThyroSeq NGS results, 12 (41.4%) were classified as positive. 10 positive nodules continued to surgical resection and the final diagnosis after thyroidectomy were: 6 malignancy (60%), 1 NIFTP (10%), and 3 benign (30%). The details are described in Table 1. ThyroSeq NGS had overall 70% PPV (7/10), 85% specificity (17/20), 100% NPV (17/17) and 100% sensitivity (7/7) in this study. Compared to Afirma Gene Sequencing Classifier (GSC), another molecular test for indeterminate thyroid nodules by cytopathology at our institution, the overall PPV and specificity for ThyroSeq NGS was 70% and 85% compared to the PPV and specificity of 69.6% and 91% for GSC.

Cases	*Final diagnosis*	*ThyroSeq NGS results*
3	Papillary thyroid carcinoma (PTC), follicular variant	Fusion: THADA/IGF2BP3
1	PTC	Mutation: BRAF V600E
1	PTC, follicular variant	Mutation: TERT C228T, NRAS Q61R
1	PTC, follicular variant	Mutation: NRAS Q61R
1	Noninvasive Follicular Thyroid Neoplasm With Papillary-Like Nuclear Features (NIFTP)	Mutation: DICE1 E1813Q
1	Follicular adenoma	Mutation: PTEN K66Rfs*33
1	Follicular adenoma	MULTIPLE CHROMOSOMAL ALTERATIONS
1	Nodular goiter	Mutation: KRAS G12C

Conclusions: ThyroSeq NGS results from the Endocrinology practice in a single institution show that PPV and specificity are similar to reports from studies at other institutions (Nikiforova et al 2018 and Kargi et al 2017). Implementation of ThyroSeq NGS may have prognostic and optimal therapeutic roles including potentially targeted therapies and finally improve patient care.

387 Pulmonary Scar Carcinomas: Cytomorphologic Features in Histologically Confirmed Cases

Sigfred Lajara[1], Humberto Trejo Bittar[2], Sanja Dacic[3], Sara Monaco[4], Liron Pantanowitz[5]
[1]University of Pittsburgh Medical Center Presbyterian Shadyside, Pittsburgh, PA, [2]Pittsburgh, PA, [3]University of Pittsburgh, Pittsburgh, PA, [4]University of Pittsburgh Medical Center, Pittsburgh, PA, [5]University of Pittsburgh, Wexford, PA

Disclosures: Sigfred Lajara: None; Humberto Trejo Bittar: None; Sanja Dacic: *Advisory Board Member*, Bayer; *Advisory Board Member*, Takeda; Sara Monaco: None; Liron Pantanowitz: None

Background: Lung carcinoma arising in association with scar tissue is a common phenomenon. Scar tissue complicates imaging studies, as well as pathologic measurement of tumor area for cancer staging. To the best of our knowledge, the cytologic findings in lung scar carcinoma (LSC) have not been described in the literature. Therefore, the aim of this study was to characterize the findings in fine needle aspirations (FNA) from histologically confirmed LSCs.

Design: A total of 53 histologically proven LSC were identified on retrospective search. Cases where an FNA was performed were selected and reviewed. The clinical and surgical pathology findings were recorded. Cytology material was further evaluated for cellularity, pattern, and presence of background stroma.

Results: Among the 14 (26%) cases identified with available FNA material, 12 (86%) were adenocarcinomas (ADC) and 2 (14%) were squamous cell carcinomas (SCC). Tumor sizes ranged from 1.3 to 4.4 cm and the amount of fibrosis in resection specimens ranged from 5-80%. Only 3 (21%) cases were hypocellular, despite extensive (up to 80%) fibrosis noted on resection. FNAs were diagnosed as positive for malignancy in 12 (86%) cases, suspicious for malignancy in 1 (7%) case, and atypical in 1 (7%) case. Whilst there was good correlation with cytology and the dominant pattern type on histology in 10 (83%) ADC cases, tumors with a papillary component on histology had more of a solid pattern on FNA. One SCC was keratinizing and the other was non-keratinizing, which both correlated with cytology. All cases had focal spindle cells, fibrous or fibroelastotic tissue fragments on smears or cell block.

Conclusions: This is the first study that describes the cytologic findings associated with lung scar carcinomas. The presence of tumor fibrosis did not negatively impact FNA sample cellularity, which is likely due to multiple excursions and selective microdissection of tumor cells by the FNA needle. The cytologic-histologic patterns correlated in most cases. Although FNA is feasible to provide a pre-operative diagnosis of carcinoma, a definitive diagnosis of LSC requires surgical resection.

388 Oncocytic or Oncocytoid? Mimics and Markers of Oncocytic Differentiation as Revealed by Molecular Profiling of Thyroid Fine Needle Aspirates Diagnosed as Oncocytic Follicular Neoplasm

Michael Landau[1], Yuri Nikiforov[1], N. Paul Ohori[2], Simon Chiosea[3]
[1]University of Pittsburgh Medical Center, Pittsburgh, PA, [2]UPMC-Presbyterian Hospital, Pittsburgh, PA, [3]University of Pittsburgh, Pittsburgh, PA

Disclosures: Michael Landau: None; Yuri Nikiforov: *Stock Ownership*, University of Pittsburgh; N. Paul Ohori: None; Simon Chiosea: None

Background: Markers of Follicular Neoplasm Oncocytic Cell Type (FNOCT) include Kallikrein 1 (KLK1) overexpression (PMID 20926528) and copy number alterations (CNAs, i.e. near-haploidization; PMID 24909752). In contrast, autonomously functioning thyroid nodules (AFTNs) with oncocytoid features (“oncocytoid mimickers”) are driven by thyroid stimulating hormone receptor (TSHR) or enhancer of zeste 1 polycomb repressive complex 2 subunit (EZH1) mutations and/or solute carrier family 5 member 5 (SLC5A5) overexpression. AFTNs and other oncocytoid mimickers can be distinguished from true oncocytic neoplasms by molecular testing of thyroid fine needle aspirates (FNAs) diagnosed as FNOCT.

Design: Consecutive thyroid FNAs over 19 months diagnosed as FNOCT and prospectively tested by a molecular panel (PMID 29345728) were identified (n=109). Cytologic and molecular findings were correlated with surgical pathology follow-up.

Results: Molecular testing identified 16 (14.7%) oncocytoid mimickers, including 12 (11%) AFTNs (with TSHR mutation +/- SLC5A5 overexpression, n=8, or EZH1 mutation, n=4), 3 (2.8%) papillary thyroid carcinomas (PTCs) (with BRAF V600E mutation), and one (0.9%) hyalinizing trabecular tumor (HTT) (with PAX8-GLIS3 fusion, PMID 30648929). 50 (46%) were confirmed as oncocytic neoplasms, based on CNAs and/or KLK1 overexpression, including 14 cases with KLK1 overexpression alone. On surgical follow-up, oncocytic differentiation was seen without CNA or KLK1 overexpression only in neoplasms with HRAS (follicular oncocytic adenoma, FOA, n=2; oncocytic follicular variant of PTC, n=1), KRAS (FOA, n=2), or NRAS mutation (FOA, n=1 and follicular oncocytic carcinoma, n=1). 36 (33%) FNOCT were negative for mutations, fusions, CNAs, and KLK1 overexpression.

Conclusions: Oncocytoid mimickers represented 15% of FNOCT and included AFTNs, PTCs, and HTT. Most (59%) FNOCT nodules show no molecular evidence of oncocytic differentiation, as judged by CNAs and/or KLK1 overexpression. Rarely, RAS mutations are associated with oncocytic differentiation in the absence of CNA and KLK1 overexpression. The absence of clonal events in the remaining FNOCT suggest that these are either driven by yet to be uncovered molecular alterations, or are non-neoplastic.

389 Comparing Anal High-Grade Cytological Categories: Histologic Outcomes and HPV Prevalence

Volha Lenskaya[1], Michael Gaisa[1], Keith Sigel[1], Yuxin Liu[2]
[1]Icahn School of Medicine at Mount Sinai, New York, NY, [2]Mount Sinai Health System, New York, NY

Disclosures: Volha Lenskaya: None; Michael Gaisa: None; Keith Sigel: None; Yuxin Liu: None

Background: Anal cytology has emerged as the primary screening test for human papillomavirus-associated anal cancer in high-risk populations. Like cervical cytology, anal cytology follows the 2001 Bethesda system that includes two high-grade categories: high-grade squamous intraepithelial lesion (HSIL) and atypical squamous cells, cannot exclude HSIL (ASC-H). However, an unofficial term "low-grade SIL cannot exclude HSIL" (LSIL-H) is also used for LSIL samples containing a few cells that are suspicious for but not diagnostic of HSIL. This study compared histologic outcomes and prevalence of high-risk human papillomavirus (HR-HPV) between these three high-grade categories.

Design: The anal dysplasia clinic database from 2015 to 2019 was searched for patients with HSIL, ASC-H or LSIL-H diagnoses on initial anal screening cytology. Only those followed with high-resolution anoscopy (HRA)-guided biopsy within 3 months were included in the

study. Cytology samples were tested for HPV16, 18 and 12 other HR-HPV types using the Cobas@ 4800 system. Two pathologists reviewed all cases and recorded the type and number of diagnostic cells in a high power field (400x).

Results: Anal cytology samples from 154 patients met inclusion criteria, including HSIL (n=66), ASC-H (n=36), and LSIL-H (n=52). Nearly all samples (98%) tested positive for HR-HPV. Table 1 shows histologic outcomes and HPV co-testing results for each group. ASC-H and LSIL-H groups revealed nearly identical incidence of AIN 2/3 on follow-up biopsy (78% and 77%) and HPV16/18 infection (61% and 63%), whereas HSIL group showed a higher incidence of AIN 2/3 (89%; p=0.15) and HPV16/18 infection (70%; p=0.63). Compared with HSIL cases, ASC-H and LSIL-H cases contained fewer diagnostic HSIL cells and primarily as the keratinized type rather than the classic type.

TABLE 1. Histologic outcomes and prevalence of high-risk HPV for three high-grade categories in anal cytology (n=154)

Cytology diagnosis	Case #	Histologic outcomes			High-risk HPV			
		AIN 3 n (%)	AIN 2 n (%)	AIN 1 n (%)	HPV16/18 n (%)	Others n (%)	Negative n (%)	
HSIL	66	26 (39)	33 (50)	7 (11)	46 (70)	19 (29)	1 (1)	
ASC-H	36	12 (33)	16 (45)	8 (22)	22 (61)	12 (33)	2 (6)	
LSIL-H	52	16 (31)	24 (46)	12 (23)	33 (63)	19 (37)	-	
Total	154	54 (35)	73 (47)	27 (18)	101 (66)	50 (32)	3 (2)	

Conclusions: Among individuals with risk factors for anal cancer, all three high-grade cytological categories have high predictive value for HPV16/18-associated anal precancerous lesions; thus, HRA referral is warranted. LSIL-H and ASC-H overlapped significantly in performance characteristics, lacking sufficient distinction to justify the creation of two separate categories. The non-Bethesda diagnosis, LSIL-H, should be discouraged to avoid redundancy and confusion in clinical management.

390 Efficacy of Performing Ziehl–Neelsen Stain Routinely on Bronchoalveolar Lavage Specimens Sent for Cytopathology Evaluation: Our Experience

Marino Leon[1], Stacy Beal[1], Julia Ross[2], Jacquelyn Knapik[3], F. Zahra Aly[3], Faisal Mukhtar[1], Peter Drew[1], Erin Weeden[4]
[1]University of Florida, Gainesville, FL, [2]University of Florida, Lake Worth, FL, [3]University of Florida College of Medicine, Gainesville, FL, [4]Fort Walton Beach Medical Center, Fort Walton Beach, FL

Disclosures: Marino Leon: None; Stacy Beal: None; Julia Ross: None; Jacquelyn Knapik: None; F. Zahra Aly: None; Faisal Mukhtar: None; Peter Drew: None; Erin Weeden: None

Background: Bronchoalveolar lavage (BAL) obtains alveolar and some bronchial material that can further be evaluated for infectious, inflammatory, and neoplastic lung diseases. Cytopathology laboratories may perform an acid fast bacteria (AFB) stain, such as Ziehl-Neelsen (ZN) on cytology preparations (i.e. cytospin slides) for identifying mycobacterial infections. Although less specific, the auramine-rhodamine fluorescent stain (ARFS) for AFB is reportedly a more sensitive, faster and less labor intensive method of screening; and the CDC currently recommends it for detecting AFB in primary patient specimens. We assessed whether our current practice of ZN staining on cytospin preparations was as effective as the ARFS performed in the microbiology laboratory.

Design: A list of all the BAL specimens from January 1, 2013 to August 16, 2018 that had a positive AFB smear identified by ARFS in the microbiology laboratory was obtained. Those BAL specimens with a corresponding sample evaluated by cytopathology using the ZN stain on a cytospin preparation were selected for the study. The reports were reviewed. The ZN stained cytopathology slides from these cases were secondarily reviewed by a pathologist.

Results: There were 91 BAL specimens with a positive ARFS in the microbiology laboratory that also had a concurrent aliquot of the BAL material sent to the cytopathology laboratory and evaluated with a ZN stain. Of those, 89 (97.8%) cases subsequently grew acid fast organisms in microbiology cultures. Of the 91 specimens, cytopathology originally reported AFB organisms in only 21 (23%) cases. Upon secondary review of the cytology cases, AFB organisms were only identified in 44 (48.4%) cases.

Conclusions: Our institutional experience shows that cytopathology examination using ZN stain is not as effective as the ARFS performed in the microbiology laboratory for identifying AFB in BAL specimens. It is therefore, reasonable to discontinue routine ZN stains performed on every BAL specimen sent for cytopathology when a specimen is sent to microbiology and examined using ARFS. The AFB stain can still be performed at the specific request of the treating clinician if clinical suspicion is high. Possible benefits may include reduction in supply costs and improved utilization of technical and professional time.

391 Molecular Testing in the Management of Indeterminate Thyroid Nodules by Fine Needle Aspiration

Wencheng Li[1], Michael Cohen[2]
[1]Wake Forest Baptist Medical Center, Winston-Salem, NC, [2]Wake Forest University, Winston-Salem, NC

Disclosures: Wencheng Li: None; Michael Cohen: None

Background: Molecular testing has been used to stratify cytologically indeterminate thyroid nodules and resolve the diagnostic uncertainty of thyroid FNA. ThyroSeq® is a comprehensive, next-generation sequencing panel of genetic markers that improves cancer diagnosis in these nodules.

Design: Thyroid nodules with indeterminate cytology (Bethesda III, IV) that underwent ThyroSeq® testing between 2016 to 2019 were retrospectively reviewed. A control cohort included cases with indeterminate cytology and no molecular testing between 2012 to 2015. Cytologic diagnosis, molecular results, and histologic data were collected.

Results: We identified 202 indeterminate thyroid nodules that underwent molecular testing (128 in Bethesda III and 74 in IV). Mutations were found in 59 nodules with mutation rates of 22.7% in Bethesda III and 40.5% in IV. In this cohort, 47 cases had surgical resection with an overall resection rate of 23.3% (47/202, 15.6% in Bethesda III and 36.5% in IV). Among the resected cases, 41 cases had positive mutation results. Thyroid cancer was diagnosed in 21 nodules with a malignancy detection rate of 10.4% (21/202, 4.7% in Bethesda III and 20.3% in IV). The histologic diagnoses of the other 26 resection cases included follicular adenoma (15 cases), nodular hyperplasia (10 cases), and non-invasive follicular thyroid neoplasm with papillary-like nuclear features (1 case). In the cohort without molecular testing, we identified 236 indeterminate thyroid nodules (158 in Bethesda III and 78 in IV). Surgical resection was performed in 127 cases with an overall resection rate of 53.8% (127/236, 46.2% in Bethesda III and 69.2% in IV). Thyroid cancer was diagnosed in 30 nodules with a malignancy detection rate of 12.7% (30/236, 6.3% in Bethesda III and 25.6 in IV). There were more papillary microcarcinomas detected (14 cases) in the cohort without molecular testing than the other cohort (2 cases).

Conclusions: Thyroid molecular testing significantly decreased the surgical resection rate (from 53.8% to 23.3%, $p<0.001$ by Fisher Exact Test) in indeterminate thyroid nodules at our institution. The thyroid cancer detection rate in the two cohorts differed slightly (10.4% vs. 12.7%), and was not statistically significant. Moreover, most of the cancers that were missed by molecular testing were papillary microcarcinomas. ThyroSeq® appears to have value in decreasing the number of patients undergoing surgery for indeterminate thyroid nodules.

392 Real-Time Polymerase Chain Reaction Crossing Point Value as Predictor of Squamous Intraepithelial Lesion

Yilan Li[1], Matthew Binnicker[2], Aimee Boerger[2], Michael Henry[2], Melanie Bois[2]
[1]Mayo Clinic, Auburn Hills, MI, [2]Mayo Clinic, Rochester, MN

Disclosures: Yilan Li: None; Matthew Binnicker: *Advisory Board Member*, Roche; Aimee Boerger: None; Michael Henry: None; Melanie Bois: None

Background: The relationship between high-risk Human Papilloma Virus (hrHPV) burden and the natural history of squamous intraepithelial lesions (SIL) remains controversial, although most studies indicate a positive correlation between the amount of virus present and disease progression. Real-time polymerase chain reaction (rtPCR) is commonly used in clinical laboratories for hrHPV testing, resulting in crossing point (Cp) data (the number of cycles needed for a test to become positive). Thus, the Cp is inversely related to, and serves as a semi-quantitative surrogate for, viral load testing. We aimed to investigate the utility of the Cp value in predicting the clinical course of hrHPV-positive patients.

Design: hrHPV-positive patients (pts) (n=734) diagnosed during pap smear co-testing at a single referral institution (2014-2015) were retrospectively identified. Salient clinical information, including initial pap smear diagnosis and follow up pathology for HPV-16 and HPV-18-positive pts, was abstracted from the medical record. Cp data from the Roche cobas 4800 rtPCR (Roche Diagnostics, Indianapolis, IN) for all hrHPV types were collated (HPV-16, HPV-18, hrHPV "other") and correlated with clinical outcome.

Results: 189 pts were positive for HPV-16 and/or HPV-18. Pts had a median age of 43 years (IQR, 22-85). Of those, 70 (37%) were positive for more than one type of HPV. Follow up data were available in 159 (84%) pts, with a median time of 1096 days (IQR, 9-1967). Among all patients, early hrHPV Cp was related to the likelihood of a positive pap smear during the follow up period (ASCUS+, p=0.0027 and LSIL+, p=0.0246). Within HPV-16-positive pts, lower HPV-16 Cp correlated with high grade SIL on follow up biopsy or cone (SIL1 vs SIL2+, p=0.0029). Limiting the scope to HPV-16-positive cervical lesions demonstrated similar results (CIN1 vs CIN2+, p=0.0021). However, the same relationship was not observed in HPV-18-positive patients (p=0.5295), possibly due to low sample size (n=22). Analysis of hrHPV "other" category is ongoing.

Conclusions: Preliminary data suggests that Cp data, a parameter readily available in many clinical laboratories, may be an indicator of clinical course in HPV-16-positive patients. Given the potential for guiding clinical management, inclusion of hrHPV Cp data in the final report should be explored and modeled in larger patient populations.

393 Cytomorphology, Immunoprofile and Outcomes of Renal Oncocytic Tumors

Xiaoqi Lin, Northwestern University, Chicago, IL

Disclosures: Xiaoqi Lin: None

Background: Hybrid oncocytic tumor (HOT) is a rare uncommon tumor that shows overlapping histologic features with renal oncocytoma (RO) and chromophobe renal cell carcinoma (ChRCC). It was originally identified in patients with the Birt-Hogg-Dube syndrome, but can also be sporadic. The comparison of cytomorphology (architectures, cell shape and size, nuclear (N) features and cytoplasmic features), immunoprofiles and outcomes of oncocytic tumors, HON, RO and ChRCC, have not been well studied.

Design: In this study, six HOTs, 34 RO and 23 ChRCCs with fine needle aspiration (FNA) and/or needle core biopsy (NCB) with touch preps were retrieved. Cytomorphology, immunoprofiles and outcomes were studied. Follow-up periods were from 3 months to 17 years.

Results: See Table 1 and 2

Among the immunomarkers tested (HP-1, CK7, CD117, PAX8, MUC1, p16, H2AFX, S100A1, CA9 CD10, AMACR, and CK20, only CK7 (diffuse positive in 67% ChRCC, vs. 0% RO or HOT, P<0.01 and <0.01, respectively), AMACR (diffuse positive in 64% RO, vs 0% in HOT and 10% ChRCC, P<0.01 and <0.01, respectively), and MUC1 (diffusely positive in 94% ChRCC vs. 55% in RO, P<0.05).

Table 1. Significant cytomorphology of RO, HOT and ChRCC.

Cytomorphology	HOT(%)	RO(%)	ChRCC(%)	P Value
Sheet	27	0	63	P2<0.01; P3<0.01
3D	33	50	75	P2<0.01
Vessel	Surround cell nest	Surround/transverse cell nest/sheet	Transverse cell sheet	P2<0.01
Cell Size	Small-large(R)	Medium-large	Small-large	P1<0.05; P2<0.01
N Size (Large)	3	0	58	P2<0.01; P3<0.01
N Pleomorphism	3	0	58	P2<0.01; P3<0.01
Prominent Nucleoli	6	17	71	P2<0.01; P3<0.05
N irregularity	3	0	71	P2<0.01; P3>0.01
N Grade	1.1 ± 0.3	1.5 ± 0.5	2.4 ± 0.8	P2<0.01; P3<0.05
Cytoplasmic Amount	Little-abundant(R)	Medium-abundant	Little-abundant	P1<0.01; P2<0.01
Delicate/Pale	1	33	26	P1<0.05; P2<0.05
Dense	29	17	70	P2<0.01; P2<0.05

3D: 3-dimensional cluster; Cell size: Large cells with abundant cytoplasm; R: rare; N: Nuclei. Yates' correction test, Chi-square test and Student T test.

P1: Comparison of oncocytoma with HOT; P2: Comparison of oncocytoma with ChRCC; P3: Comparison of HOT with ChRCC.

Table 2. Outcomes of RO, HOT and ChRCC after cytology biopsies

Outcomes	RO(%)	HOT(%)	ChRCC(%)	P Value
Nephrectomy	6	67	67	P1< 0.01; P2<0.01
Cyroablation	6	0	13	
Surveillance	82	33	13	P1< 0.01; P2<0.01
Deceased	1(stroke)			
N/A	2.9			
Metastasis	0	0	26	P1< 0.01; P2<0.01

Conclusions: 1. Large sheets of tumor cells, 3D clusters, transversing vessels, higher nuclear grade (prominent nucleoli, irregular nuclei, large nuclei and nuclear pleomorphism) and large cells with abundant pale cytoplasm or dense cytoplasm are helpful cytologic features to distinguish ChRCC from RO or HOT. Only cell size, cytoplasm amount and pale/clear cytoplasm are helpful to distinguish RO from HOT. 2. Among the tested markers, only diffuse positivity for CK7, AMACR and MUC1 are useful to distinguish RO, HOT and ChRCC. 3. Therefore, it is important to combine all the information of cytologic and histologic features and immunoprofiles as well as imaging studies to render definitive diagnosis of renal oncocytic tumors. 4. It is critical to make definitive diagnosis of renal oncocytic tumors in renal biopsies to order to guide clinicians to appropriately manage their patient, surgery resection, ablation, and active surveillance.

394 Clinical and Cytopathological Features of Suspected Thyroglossal Duct Cysts and Neoplasms Arising from Them: A Large Series from a Referral Cancer Center

Zhonghua Liu[1], Tieying Hou[2], Qiong Gan[2], Savitri Krishnamurthy[2]
[1]The University of Texas MD Anderson Cancer Center, Austin, TX, [2]The University of Texas MD Anderson Cancer Center, Houston, TX

Disclosures: Zhonghua Liu: None; Tieying Hou: None; Qiong Gan: None; Savitri Krishnamurthy: None

Background: Thyroglossal duct cysts (TGDC) are the most common congenital midline neck cystic lesions. Most TGDC are benign, but some give rise to neoplasms. Recognizing the cytomorphologic features of TGDC and their mimics is important for clinical management. The primary objective of our study was to elucidate the clinicopathologic features of a large series of suspected TGDCs and neoplasms arising from these cysts.

Design: We studied the clinicoradiological and cytopathological features of 69 cases of ultrasonography-guided fine needle aspiration (US-FNA) for clinically suspected TGDCs or neoplasms arising from TGDCs at our institution between 2002 and 2019. Cytopathological findings on aspiration, cytospin smears, and cell block sections were correlated with surgical follow-up and/or imaging studies.

Results: The median age of patients was 59 years (range, 1-89 years) and the median lesion size was 1.6 cm (range, 0.5-5.1 cm). On US examination, 43 lesions (62%) were recognized as cystic lesions with septation without vascular flow, 21 (31%) as hypoechoic nodules, and 5 (7%) as mass-like lesions.

By cytopathological examination, 65 lesions (94%) were categorized as benign and 4 (6%) as malignant. Of the 65 benign lesions, the common findings are proteinaceous material (62%), histiocytes (58%) and colloid (40%). Other features include squamous cells (37%), columnar cells (32%), thyroid follicular cells (14%), inflammatory cells (11%), and multi-nucleated giant cells (5%).

FNA diagnoses based on the cytopathological and imaging findings included TGDC in 50 patients (73%), TGDC or mimics in 13 patients (19%), colloid nodule in 1 patient, and thyroiditis in 1 patient. Surgical resection performed in 13 patients confirmed TGDC in 6 patients, epidermal inclusion cyst in 2 patients, thyroiditis in 1 patient and carcinoma in 4 patients including 2 squamous cell carcinoma (SCC) and 2 papillary thyroid carcinoma (PTC). In 2 of 4 patients (1 SCC and 1 PTC), surgical excision confirmed that the lesions originated from TGDCs.

Table 1: Clinical and pathological features of 69 cases	
Total specimen	69
Age median (range)	59 (1-89)
Sex	
Female	31
Male	37
Size: median (range) in cm	16 (0.5 - 5.1)
FNA diagnosis	
TGDC	50 (73%)
TGDC and other mimics	13 (19%)
PTC	2 (3%)
SqCC	2 (3%)
Colloid nodule	1 (1%)
Thyroiditis	1 (1%)
Surgical resection	Total 13
TGDC	6
Epidermoid inclusion cyst	2
SqCC	2
PTC	2
Thyroiditis	1
Histology of benign	Total 65
Proteinaceous fluid	40 (62%)
Histiocytes	38 (58%)
Colloid	26 (40%)
Squamous cells	24 (37%)
Columnar cells	21 (32%)
Thyroid follicular cells	9 (14%)
Inflammation	7 (11%)
Giant cells	5 (8%)
US finding	
Cystic lesion with septation, w/o blood flow	43 (62%)
Hypoechoic nodule	21 (31%)
Mass-like lesion	5 (7%)

Conclusions: Cytopathological features in conjunction with imaging findings allowed a definite diagnosis of TGDC in most patients (73%) with clinically suspected TGDCs. The presence of abundant mature squamous cells or thyroid follicular cells with or without colloid and/or lymphocytes alone allowed a differential diagnosis of TGDC and its mimics in 19% of patients. US-FNA findings could not be used to distinguish primary SCC or PTC arising from TGDCs from metastatic tumors.

395 Developing and Validating Multiplex Immunofluorescence Panels for Immune-Profiling of Non-Small Cell Lung Cancer using Cytological Cell-Blocks

Maria Lozano[1], Marta Abengozar[2], María Alvarez[3], Laura Garcia Tobar[3], Allan Argueta[3], Maria Villalba Esparza[4], José Echeveste[5], Carlos de Andrea[4]

[1]University of Navarra, Pamplona, Spain, [2]University of Navarra, Zizur Mayor, Spain, [3]University of Navarra, Pamplona, Navarra, Spain, [4]Universidad de Navarra, Pamplona, Navarra, Spain, [5]Clinica Universidad de Navarra, Pamplona, Navarra, Spain

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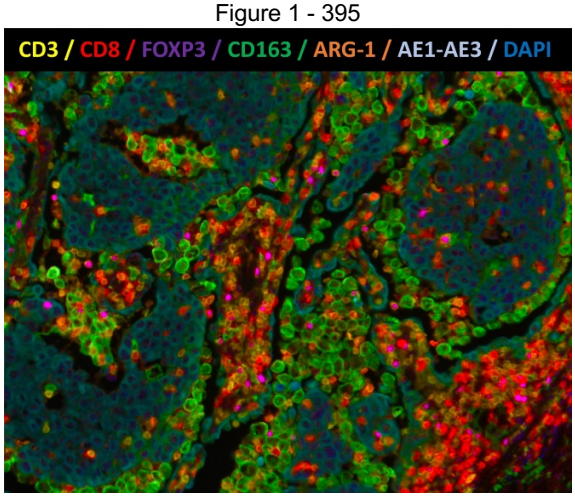
Background: Understanding the immune contexture of non-small cell lung cancer (NSCLC) is important for the designing of effective anticancer immunotherapies. The development of multiplex immunoassays is critical in lung cancer, where up to 70% of patients are diagnosed at advanced stages and tissue biopsies are often limited or cannot be taken. Multiplex immunofluorescence assays (mIF) allow the characterization of the tumor-infiltrating immune cells and their interactions, both across and within immune subtypes. Here we describe the development and validation of a MIF assay in five paired cytological cell-blocks and surgical resection specimens.

Design: Five paired cytological cell-blocks and surgical resection specimens from NSCLC were tested with MIF assays designed to detect expression of key immune cell markers such as CD3, CD8, FOXP3, CD163 and arginase-1. Pan-cytokeratin was used to detect the NSCLC cells. Fluorescence images were acquired on the Vectra Polaris platform (Perkin Elmer). Analysis of the data was performed with inForm software (Perkin Elmer).

Results: We showed that although the paired NSCLC samples shared similarities in their immunological/inflammatory features, they also display significant heterogeneity among lesions. In both cytological cell-blocks and resection specimens, the mIF assay showed a uniform,

specific, and correct staining pattern and with similar patterns of expression and specific co-localization of the immune cell markers: cytotoxic T cells (CD3 plus CD8 positive); helper T cells (CD3 minus CD8 positive), regulatory T cells (CD3 plus FOXP3 positive minus CD8 positive. Macrophage showed specific expression of CD163. In both samples type, macrophage M2 polarization was found surrounding the pan-cytokeratin positive tumor cells. Some of the CD163 positive macrophages showed arginase-1 expression, suggesting that these might be immune suppressive macrophages (M2).

Figure 1 - 395



Conclusions: The implementation of mIF in cytological cell-blocks is feasible and the staining pattern is similar to the one found on the paired surgical resection samples. Cytological cell-blocks offer several new opportunities for innovative digital pathology analysis giving insight into the spatial distribution of distinct cell types based on the co-expression of key molecules.

396 Feasibility, Reliability and Therapeutic Implications of Cytological Stained Smears as a Source of Starting Material for Next-Generation Sequencing-Based Molecular Testing in NSCLC Patients

Maria Lozano[1], Marta Abengozar[2], María Alvarez[3], José Echeveste[4], Eva Helena Royuela[5], Laura Garcia Tobar[3], Jose Joaquin Paricio[3], Allan Argueta[3], Guillermo Garcia Porrero[4], Marcela Moreno[6], Nerea Gómez[4], Carlos de Andrea[5]
[1]University of Navarra, Pamplona, Spain, [2]University of Navarra, Zizur Mayor, Spain, [3]University of Navarra, Pamplona, Navarra, Spain, [4]Clinica Universidad de Navarra, Pamplona, Navarra, Spain, [5]Universidad de Navarra, Pamplona, Navarra, Spain, [6]Universidad de los Andes, Santiago de Chile, Chile

Disclosures: Maria Lozano: None; Marta Abengozar: None; María Alvarez: None; José Echeveste: None; Eva Helena Royuela: None; Laura Garcia Tobar: None; Jose Joaquin Paricio: None; Allan Argueta: None; Guillermo Garcia Porrero: None; Marcela Moreno: None; Nerea Gómez: None; Carlos de Andrea: None

Background: Advances in precision oncology are trending toward the interrogation of limited amounts of genomic material to guide clinical and therapeutic decisions. Up to 70% of patients with NSCLC are diagnosed at advanced stages and tissue biopsies often cannot be taken. With increasing requests for the evaluation of prognostic and predictive molecular biomarkers, great attention must be paid to the preanalytical issues regarding sample quality and DNA/RNA yield from cytological preparations. Although cytology samples provide a high quality material for molecular testing, molecular cytopathology is not yet well known or widely used.

The aim of this study was to evaluate the feasibility and efficacy of cytological stained smears from NSCLC patients for next generation sequencing (NGS), as well as the impact on treatment with therapeutic targets.

Design: We examined 52 fine-needle aspiration (FNA) cytology samples (45 direct smears, 7 cell-blocks) from 52 patients. Rapid on-site evaluation (ROSE) was performed in all procedures in order to guarantee quality of samples for NGS. DNA from Papanicolaou (50) or Diff-Quick (2) stained smears was extracted and sequenced using the Ion Torrent NGS platform with two assays: 41 with Oncomine Focus Assay (OFA) and 11 with Oncomine Comprehensive Assay (OCA).

Results: ROSE maximized all cytology samples for molecular testing. High-quality DNA ($\bar{x}$ [DNA]: 43.75 ng/µl and $\bar{x}$ [RNA]:88.74 ng/µl; SD: 22.46) was extracted from >1500 intact cells from all smears. Mutations were found in 41 cases (78.8%), 30 with OFA, and 11 with OCA. In 33 cases (63.5 %) these alterations were clinically relevant, and in 16 cases (30.8%) they were pharmacologically actionable. Seven patients (13.5%) received targeted treatment.

No statistically significant differences between both gene panels were found regarding detection of clinically relevant mutations, actionable mutations and patients who received targeted treatment (p= 0.98, p= 0.53, and p=0.13)

Conclusions: Stained cytology smears are a reliable source for high-quality DNA and RNA for NGS analysis in NSCLC patients. No all patients with actionable molecular alterations received targeted treatment. Both NGS assays gave the same data regarding clinically relevant alterations. Hence, larger panels could not be necessary for limited samples. Further studies are needed. It is critical that cytopathologists become familiar with the variables that can affect test results and embrace the goal of excellence in sample quality.

397 Feasibility of PD-L1 Expression in Cytological Stained Smears: Comparison with Cellblocks and Relationship with the Outcomes of NSCLC Patients Treated with Check-Point Inhibitors

Maria Lozano[1], Laura Garcia Tobar[2], Marta Abengozar[3], José Echeveste[4], Ignacio Ortego Zabalza[4], María Alvarez[2], Jose Joaquin Paricio[2], Allan Argueta[2], Guillermo Garcia Porrero[4], Nerea Gómez[4], Carlos de Andrea[5]
[1]University of Navarra, Pamplona, Spain, [2]University of Navarra, Pamplona, Navarra, Spain, [3]University of Navarra, Zizur Mayor, Spain, [4]Clinica Universidad de Navarra, Pamplona, Navarra, Spain, [5]Universidad de Navarra, Pamplona, Navarra, Spain

Disclosures: Maria Lozano: None; Laura Garcia Tobar: None; Marta Abengozar: None; José Echeveste: None; Ignacio Ortego Zabalza: None; María Alvarez: None; Jose Joaquin Paricio: None; Allan Argueta: None; Guillermo Garcia Porrero: None; Nerea Gómez: None; Carlos de Andrea: None

Background: Programmed cell death-ligand 1 (PD-L1) protein expression is a predictive biomarker of response to programmed death 1 (PD-1) and PD-L1 immune checkpoint inhibitors in non-small-cell lung cancer (NSCLC). Up to 70% of patients with NSCLC are diagnosed at advanced stages and tissue biopsies often cannot be taken. Very few studies of PD-L1 expression in NSCLC cytology samples has been reported to date, and to our knowledge no one with treatment correlations. The aim of this study was to evaluate the feasibility and efficacy of PD-L1 quantification in cytological samples including cell blocks and smears, as well as evaluate the clinical response to immunotherapy agents based on PD-L1 expression on cytological samples.

Design: We examined 153 cytological samples (104 smears plus 49 cellblocks) from 104 NSCLC patients. PD-L1 expression was analyzed using the SP263 Roche' Ventana Assay. Tumor proportion score (TPS) was reported in every case by the two pathologist following the IASLC recommendations. Smears containing ≥ 100 tumor cells were considered adequate for the analysis. We also analyzed the response to treatment in those patients who received immunotherapy.

Results: Our series includes 83 adenocarcinomas, 17 squamous cell carcinomas, 3 LCNC, and 1 adenosquamous carcinoma. 58 (55%) patients showed PD-L1 positivity: 7- 1%; 27 ->1-49%; 24- ≥ 50%. In the 49 cases from we had paired smears and cellblock the concordance was of 77.5%.

Thirty six patients received immunotherapy. PD-L1 expression and clinical response are shown in Table 1.

Response % PD-L1 expression	Complete	Parcial	Stable disease	Progression	Lost of Follow-up
0	1	3	3	6	0
1	0	1	1	3	0
>1 -49	0	1	0	2	1
≥ 50	1	6	3	4	0
Total	2	11	7	15	1

Conclusions: Quantification of PDL-1 expression is feasible and reliable on cytological specimens, both cell blocks and smears. Concordance between smears and cellblocks is high. Response to immunotherapy is variable as shown in the literature. Further studies are needed.

398 Risk Stratification of Thyroid Nodules Suspicious for Medullary Thyroid Carcinoma and the Bethesda System for Reporting Thyroid Cytopathology

Ryan Lu[1], Daniel Miller[2], Zahra Maleki[3]
[1]Department of Pathology, The Johns Hopkins Medical Institutions, Baltimore, MD, [2]Johns Hopkins Pathology, Baltimore, MD, [3]Johns Hopkins Hospital, Baltimore, MD

Disclosures: Ryan Lu: None; Daniel Miller: None; Zahra Maleki: None

Background: Medullary thyroid carcinoma (MTC) is a malignant neuroendocrine neoplasm derived from the perifollicular C cells of the thyroid comprising 1-2% of thyroid carcinomas. Its initial presentation can be as a thyroid nodule or a neck mass and fine needle aspiration (FNA) is routinely performed to evaluate these masses. However, MTC can possess diagnostic challenges on FNA biopsy due to variable cytomorphology. The aim of this study is to investigate risk stratification of thyroid nodules suspicious for MTC in the Bethesda System for Reporting Thyroid Cytopathology (BSRTC).

Design: After institutional approval, the electronic data in a large academic institution was retrospectively searched for any thyroid FNA cases with MTC in the final reports during 2006-2018 period. Only cases with surgical follow-up were included. The cytopathology diagnosis, corresponding histology diagnosis, and ancillary studies such as immunohistochemistry (IHC) along with patients' demographics were recorded. The BSRTC were applied for all cases based on their cytopathology report.

Results: A total of 106 cases were included (46 males and 100 females) with mean age of 52.9 years (17-85 years). The number of histology confirmed MTC cases for each BSRTC category was as follows: III, 1/5, IV, 3/13, V, 12/21 and VI, 59/67, respectively (Table.1). Combined MTC and papillary thyroid carcinoma (PTC) was reported in seven histology cases. IHC is applied on large number of histology cases as well as on cytology specimens. Risk of malignancy was 60%, 92.3%. 100%, and 97% for the BSRTC categories of III, IV, V, VI, respectively. Multinodular Hyperplasia, Noninvasive Follicular Thyroid Neoplasm with Papillary-like Nuclear Features (NIFTP), follicular adenoma with Hurthle cell change, and a fibrosing inflammatory process were benign conditions masquerading MTC on aspirated material. PTC, anaplastic thyroid carcinoma, parathyroid carcinoma, and neuroendocrine neoplasms both low grade and high grade were malignant neoplasms mimicking MTC.

Bethesda	IHC on cytology	Surgical diagnosis	IHC on histology	ROM
III (n=5) (M:F, 1:4)		MTC (n=1) MTC and PTC (n=2) Multinodular Hyperplasia (n=1) Noninvasive Follicular Thyroid Neoplasm with Papillary-like Nuclear Features (n=1)	Positive for Calcitonin (n=1)	60% (3/5)
IV (n=13) (M:F, 2:11)	Chromogranin Positive (n=1) Synaptophysin Positive (n=2)	MTC (n=3) PTC (n=3) Follicular variant of PTC (n=1) Low grade epithelial neoplasm with neuroendocrine features (n=1) Hurthle Cell Carcinoma (n=1) High grade NEC(n=1) Anaplastic Thyroid Carcinoma (n=1) Parathyroid carcinoma and PTC (n=1) Follicular Adenoma with Hurthle cell change (n=1)	Calcitonin Positive (n=1) Chromogranin Positive (n=1) Synaptophysin Positive (n=1) TTF-1 Positive (n=1) Thyroglobulin Negative (n=1) Thyroglobulin Positive (n=1)	92.3% (12/13)
V (n=21) (M:F, 10:11)	Calcitonin Positive (n=3) Chromogranin Positive (n=2) CEA Positive (n=1) TTF-1 Positive (n=1) Synaptophysin Positive (n=1)	MTC (n=12) MTC and PTC (n=2) PTC (n=5) Hurthle cell carcinoma (n=1) Follicular Adenoma with Papillary Microcarcinoma (n=1)	Calcitonin Positive (n=10) Chromogranin Positive (n=6) Synaptophysin Positive (n=5) CEA Positive (n=4) TTF-1 Positive (n=4) Thyroglobulin Negative (n=4) Thyroglobulin Positive (n=3)	100% (21/21)
VI (n=67) (M:F, 23:44)	Calcitonin Positive (n = 37) Chromogranin Positive (n=14) Thyroglobulin Negative (n=13) TTF-1 Positive (n=9) Synaptophysin Positive (n=9) CEA Positive (n=8) AE1/AE3 Positive (n=6) Thyroglobulin Positive (n=3)	MTC (n=59) MTC and PTC (n=3) Small Cell Carcinoma (n=1) PTC (n=3) Fibrosing Inflammatory Process (n=1)	Calcitonin Positive (n=23) Synaptophysin Positive (n=7) Chromogranin Positive (n=6) Thyroglobulin Negative (n=6) TTF-1 Positive (n=5) CEA Positive (n=4) AE1/AE3 Positive (n=3)	97% (65/67)

Table 1. Application of Bethesda system for Reporting Thyroid Cytopathology in cases suspicious for medullary thyroid carcinoma, histologic correlation and immunohistochemistry used in cytology and histology specimens.

Footnote: IHC: immunohistochemistry; MTC: Medullary Thyroid Carcinoma; PTC: Papillary Thyroid Carcinoma; NEC: Neuroendocrine Carcinoma; III: Atypia of undetermined significance; IV: Follicular neoplasm or suspicious for a neoplasm; V: Suspicious for malignancy; VI: Malignant.

Conclusions: Our study confirms that the BSRTC is a reliable tool for reporting MTC and application of IHC improves the diagnostic accuracy on cytology specimens. Moreover, IHC application in large number of histology cases indicates that the diagnosis of MTC remains a diagnostic challenge due to its variable cytomorphology. There are combined cases of MTC and PTC which requires extra attention and add to the complexity of these entities.

399 The Cost Effectiveness and Outcome Analysis of Cytology Pap Tests with Cotesting HPV and a Three-Year Follow-up on NILM/HPV+ Group in a Tertiary Care Setting

Renuka Malenie[1], Fan Lin[1], Michele Zelonis[1], Haiyan Liu[1]
[1]Geisinger Medical Center, Danville, PA

Disclosures: Renuka Malenie: None; Fan Lin: None; Michele Zelonis: None; Haiyan Liu: None

Background: High-risk human papillomavirus (HPV) testing was a reflex test for decades. Since the publication of consensus guidelines in 2006, cervical cancer screening cytology (Pap test) with regardless HPV testing (cotesting) becomes part of the management options. However, there are limited data on HPV status for cytology NILM category, and only few published studies to analyze the outcomes and financial impact of the Pap cotesting cases. In this study, we retrospectively searched our pathology data base for Pap cotesting cases in 2016 with a 3-year followup results (surgical biopsy and/or cytology) for HPV+ cases; the data was analyzed.

Design: A retrospective search of our pathology data base for Pap test with regardless HPV testing in 2016 was performed. The 3-year followup results for all HPV+ cases were recorded and analyzed.

Results: The total Pap cases in 2016 were 30,255. Among those, 6,458 of cotesting cases were identified, including 884 HPV+ (13.7%) and 5,574 HPV- cases (86.3%) by HPV status; 5,945 NILM (92.1%) and 513 abnormal cases (7.9%) by cytology diagnosis. The 3-year followup results and cytology diagnosis for HPV+ cases are summarized in Table 1. Overall, cytology NILM accounted for 62% (548/884) of HPV+ cases. 39.6% (350/884) of HPV+ cases in all cytology categories had benign followups, with the most in NILM/HPV+ group (48.1%, 264/548). Within the NILM/HPV+ group, 14.4% (79/548) had dysplasia on 3-year followup, only 2.9 % (16/548) being high-grade (HG) dysplasia. Among the 5,574 HPV- cases, 5,397 cases belong to cytology NILM category. Each HPV testing costed $295 at our institution; the total expense to identify 16 HG dysplasia in cytology NIML group by HPV testing alone was $1,753,775 ($295/per HPV test x 5,945 NILM) in 2016.

Table 1. Three-Year Followup Results and Cytology Categories for 884 HPV+ Cases

Cytology/Followup (n, %)	Benign (%)	Dysplasia LG (%)	Dysplasia HG/CA (%)	No Followup (%)
NILM (n=548, 62%)	264 (48.1)	63 (11.5)	16 (2.9)	205 (37.4)
ASCUS (n=174, 19.7%)	62 (35.6)	51 (29.3)	35 (20.1)	26 (14.9)
ASC-H (n=42, 4.8%)	2 (20.2)	5 (11.9)	29 (69.0)	6 (14.3)
LSIL (n=78, 8.8%)	21 (26.6)	37 (47.4)	9 (11.5)	11 (13.9)
HSIL (n=39, 4.4%)	1 (2.6)	4 (10.3)	28 (71.8)	6 (15.4)
Squamous Cell CA (n=3, 0.3%)	0 (0)	0	3 (100)	0 (0)
Total (n=884)	350 (39.6)	160 (18.1)	120 (13.6)	254 (28.7)

Conclusions: Our preliminary data demonstrated that 16 cases of HG squamous dysplasia were identified on cervical biopsies within a 3-year followup, which accounted for 2.9% (16/548) in the NILM/HPV+ group, and only 0.27% (16/5945) in the total NILM cases. The total cost to perform HPV testing in NILM group was $1,753,775 ($295/per HPV test x 5,945 NILM). In addition, a significant potentially false positive HPV testing of 48.1% in the NILM group was noted. Therefore, the cost effectiveness of the current cotesting practice may deserve attention for further investigation.

400 Correlation between Cytology and Histopathology of Endobronchial Ultrasound-Guided Transbronchial Needle Aspiration; A Retrospective Study

Raima Memon[1], Allison Wrenn[2], Isam Eltoum[1]
[1]The University of Alabama at Birmingham, Birmingham, AL, [2]UAB Hospital, Birmingham, AL

Disclosures: Raima Memon: None; Allison Wrenn: None; Isam Eltoum: None

Background: Lung staging for carcinoma need to be complete and accurate for proper management. Endobronchial ultrasound-guided transbronchial needle aspiration (EBUS-FNA) is now the procedure of choice for because it is minimally invasive and inexpensive compared to mediastinoscopy. In this study, we try to determine the frequency of discrepancies between EBUS-FNA and the histologic diagnoses and assess the reasons for these discrepancies.

Design: In this retrospective study, we reviewed all the cases were staged for lung neoplasm using EBUS-FNA in the period 2010-2018. The cytology results were then compared to any histopathologic finding in subsequent biopsies or resection specimens. We define benign as lesion with negative and atypical results and malignant as lesions with suspicious and positive results. Discrepancies were then classified as major when a neoplastic process interpreted as benign and vice versa and minor when the difference concern neoplasm classification but not its presence. Reasons for discrepancies were classified as sampling or interpretation errors for either cytology or histology.

Results: 222 patients who underwent EBUS-FNA procedure during the study period (50.6 % Female, 49.4% male, mean age 55 years), with a total of 1109 samples from either lymph node or primary lung lesion. 83 (38%) patients had both cytology and histopathological tissue diagnosis available. 202 cytology and histology samples from these patients were reviewed including both sampling of lymph nodes and lung lesion. There were 52 (26%) discrepant cases (16 (31%) false positive and 36 (69%) false negative). There were 51 major discrepancies and 1 minor. 65% discrepancies were in primary lesion vs 35 % in lymph node metastasis. Based on final resection and clinical follow-up, the majority (40 (77%)) were sampling error. Currently we are reviewing each pair of discrepancy to determine the adequacy level of these cases based on published criteria.

	Non-discrepant Cases		Discrepant Cases		
	True positive (TP)	True Negative (TN)	False positive (FP)	False Negative (FN)	Total
Primary lung lesion	6 (19.3%)	1 (9%)	10 (90.9%)	25 (80.6%)	42
Lymph node	4 (26.6%)	139 (95.8%)	6 (4.1%)	11 (73.3%)	160
Total	10	140	16	36	202

% are calculated using sensitivity and specificity calculations.

Conclusions: False negative/positive are frequent in EUS-FNA. Sampling error explains the majority of discrepant cases. There is a need to better define adequacy for EBUS-FNA.

401 Toluidine Blue or Diff-Quik: Ideal for ROSE Telecytology?

Alejandro Mendoza, Oakland, CA

Disclosures: Alejandro Mendoza: None

Background: Successful pathologic diagnosis after image-guided fine needle aspiration (FNA) depends on several factors including nature of the lesion, aspirator skill, and availability of rapid on-site evaluation (ROSE). ROSE has proven to improve the sensitivity and diagnostic yield of FNA. There are, however, several barriers that hinder the availability of ROSE during the FNA procedure. The need for experienced on-site professionals and the lack of proper billing for ROSE are some factors that limit its availability. Remote evaluation of FNA aspirate through telecytology is a solution. Telecytology in ROSE is an emerging trend. However, the ideal staining method suitable for ROSE telecytology has not yet been fully studied. The rapid stain for ROSE is the rate-limiting factor for achieving ideal ROSE images. We investigated the rapid staining methods most commonly used for ROSE in our institution. We investigated whether Toluidine blue (TB) or Diff-Quik (DQ) is ideal for ROSE telecytology.

Design: Over one hundred discarded and de-identified cytology body fluid specimens were screened for cellularity and storage time. Eleven cases with adequate cellularity and less than two weeks old were included. Three slide smears were prepared from each case; two smears were fixed with alcohol for Pap stain and TB; one smear was air-dried for DQ. Pap stain was used as a reference in the study. DQ and TB were compared using 3 parameters: image quality, efficiency, and cost-effectiveness. Image quality was graded according to five criteria: presence of residual background staining, cytoplasmic detail, nuclear membrane, chromatin texture, and staining of nucleoli. Each image quality criterion is given a score of 1 to 3 (Table 1). For efficiency, we compared the total time to perform each staining method. For cost-effectiveness, we compared the total direct cost of each rapid stain. The results were recorded and compared.

Results: TB is more cost-effective costing $12.00 to perform per slide versus $27.00 for DQ (Figure 2). TB is more efficient averaging 10 seconds to perform per slide versus 120 seconds for DQ (Figure 2). DQ results in better cytoplasmic detail and leaves a cleaner background (Figure 1).

	SCORES		
CRITERIA	**3**	**2**	**1**
1. Nuclear Membrane	distinct	some detail	not distinct
2. Chromatin texture	distinct	some detail	not distinct
3. Nucleoli	distinct	some detail	absent/not distinct
4. Cytoplasmic detail	distinct	some detail	not distinct
5. Residual background stain	clean/absent	mild/moderate presence	dirty

Table 1. Criteria for grading image quality. Each criterion is graded from 1 to 3. The higher the score, the better is the staining quality.

Figure 1 - 401

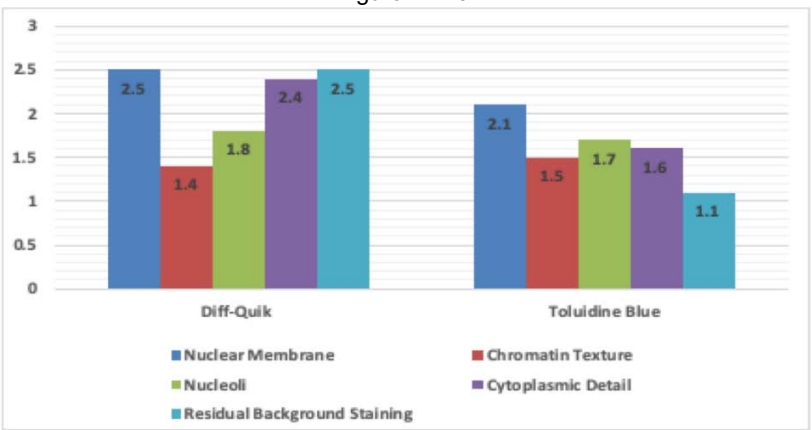


Figure 1. Image quality average scores of DQ and TB on 5 criteria (nuclear membrane, chromatin texture, nucleoli, cytoplasmic detail, residual background staining.

Figure 2 - 401

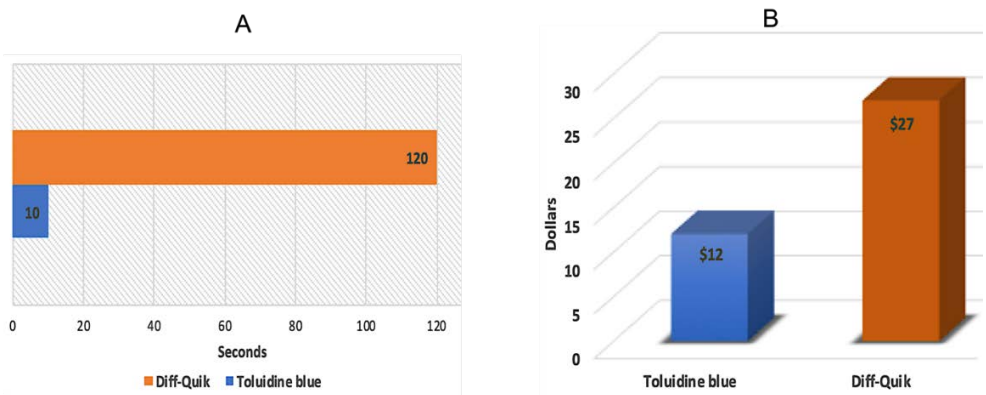


Figure 2. A. Average time to perform ROSE using TB or DQ per slide. **B.** Average direct cost to perform TB or DQ per procedure.

Conclusions: DQ provides a good image quality but is not efficient and cost-effective. TB is efficient and cost-effective but does not provide an excellent image quality. A different rapid stain that is efficient, cost-effective, and provides excellent image quality should be explored.

402 Health Disparities in Cervical Cancer: Prevalence of High Risk HPV and Cytologic Diagnoses According to Race

Daniel Miller[1], Christopher Morris[2], Zahra Maleki[3], Marissa White[4], Erika Rodriguez[4]
[1]Johns Hopkins Pathology, Baltimore, MD, [2]Johns Hopkins Medical Institutions, Baltimore, MD, [3]Johns Hopkins Hospital, Baltimore, MD, [4]Johns Hopkins University School of Medicine, Baltimore, MD

Disclosures: Daniel Miller: None; Christopher Morris: None; Zahra Maleki: None; Marissa White: None; Erika Rodriguez: None

Background: A causal link between infection with high-risk human papilloma virus (HRHPV) and the development of cervical squamous cell carcinoma is well-established. It has been reported that Black women have a higher incidence of HPV-associated cervical cancer than Caucasians. Here we report HPV testing results for a 24 month period and compare Black to Caucasian women. To assess potential effects of vaccination and updated screening guidelines, we compare cytology diagnoses in 21 – 25 year olds in 2011 and 2017.

Design: We evaluated all PAP specimens at a tertiary medical center across all ages for 24 months beginning in 2017. For each specimen, we determined results of HRHPV testing. We then assessed these specimens according to patient race groups including providers' designation of 'Black' (B) or 'Caucasian' (C). Next, we assessed cytologic diagnoses in B and C cohorts in patients aged 21 – 25 in 2011 and 2017.

Results: HPV genotype results were reviewed for 19468 specimens, including 8515 B and 10953 C women. 15% (2974) tested positive for HRHPV genotypes. The most common HPV genotype(s) detected was non-16/18 HRHPV, comprising 78% (2334/2974) of the positive results. Non-16/18 HRHPV was more common in B (1309) compared to C (1025) women, representing 11% and 7% respectively of all patients who underwent Pap testing ($p<0.01$). HPV16 was more common in C compared to B women (192 vs 99) while HPV18 was detected more frequently in B compared to C women (76 vs 46). However, we found that there was no significant difference in total HRHPV infection rates according to race (52% for B and 54% for C women).

In comparing cytology results in 2011 to 2017, we observed a lower incidence of high grade lesions (OR for HSIL is 0.36 [95% CI 0.15-0.86]). Overall, the distribution of cytologic diagnoses were similar in B, compared to C women. Among 21-25 year old B women 7.11% (73/1041) *vs.* 4.46% (36/808) of C women had diagnoses of LSIL ($p=0.01$). Of patients infected with non-16/18 HRHPV, ASCUS was the most common diagnosis in both B & C women; and the difference compared to non-HPV infected patients was statistically significant ($p<0.01$).

High-risk HPV DNA testing in cervical cytology specimen in 21-25 years old women 2011 and 2017

Diagnosis	2011 (n=669, 100%)			2017 (n=331, 100%)		
	HPV positive	HPV negative	% positive	HPV positive	HPV negative	% positive
	n=341 (51%)	n=328 (49%)		n=172 (52%)	n=159 (48%)	
NILM	66	223	23%	37	109	25%
ASC-US	198	90	69%	109	47	70%
ASC-H	32	11	74%	10	1	91%
LSIL	29	3	91%	12	1	92%
LSIL-H	8	0	100%	3	0	100%
HSIL	7	0	100%	1	0	100%
AGC	1	1	50%	0	1	0%

Abbreviations: AGC, atypical glandular cells; ASC-H, atypical squamous cell cannot exclude HSIL; ASC-US, atypical squamous cells of undetermined significance; HSIL, high-grade squamous intraepithelial lesion; LSIL, low-grade squamous intraepithelial lesion; LSIL-H, low-grade squamous intraepithelial lesion, high-grade cannot be excluded; NILM, negative for intraepithelial lesion or malignancy

Figure 1 - 402

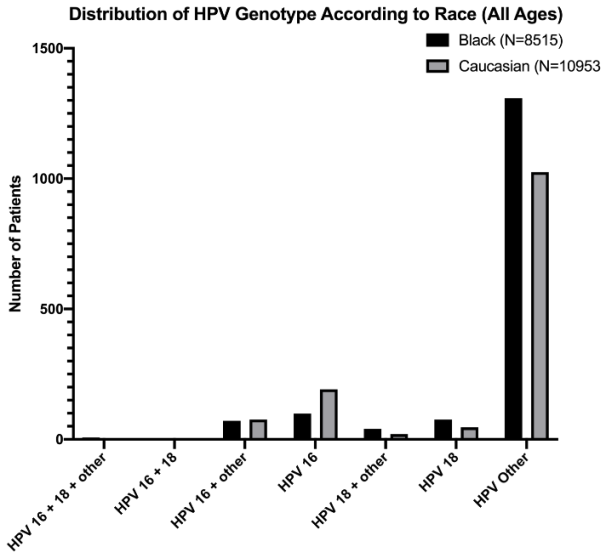
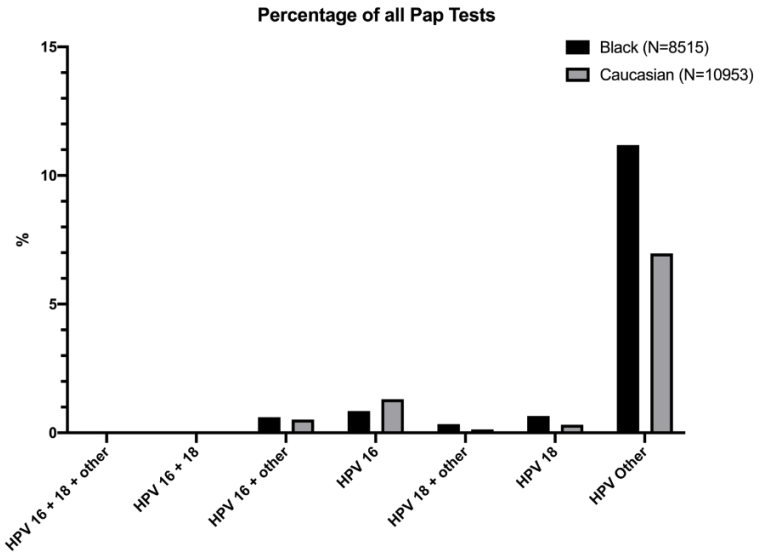


Figure 2 - 402



Conclusions: These data suggest that national black cervical cancer disparities are due to factors such as socioeconomic barriers to healthcare access or differences in genotype infection. Better understanding prevalence of specific HPV genotype infection rates in non-Caucasian patients may help guide future improvement of HPV vaccination coverage and screening guidelines.

403 Atypical Squamous Cells in Urine Cytology: A Clinical/Cytological Review with Histopathological Correlations

Fatima Mir[1], Prih Rohra[1], Matthew Vega[1], Paolo Gattuso[2], Lin Cheng[3]
[1]Rush University Medical Center, Chicago, IL, [2]Rush University Medical Center, Burr Ridge, IL, [3]Rush University Medical Center, Hinsdale, IL

Disclosures: Fatima Mir: None; Prih Rohra: None; Matthew Vega: None; Paolo Gattuso: None; Lin Cheng: None

Background: Urine cytology is a noninvasive screening tool mainly for high-grade urothelial carcinomas. Mature squamous cells are commonly seen in urine, especially in female patients. However, the presence of atypical squamous cells (ASCs) in urine is rare. Here we performed a retrospective study to evaluate the various etiologies of ASCs in urine and to better understand its significance.

Design: We searched our cytology database from 1979-2018 to identify cases of urine cytology with presence of atypical squamous cells. Available follow-up biopsies and clinical data were also reviewed.

Results: In the 39-year study period, 53 cases of ASCs were identified in 4759 urine cytology specimen. There were 23 males and 30 females with age ranging from 28-88 years. In 29/53 (55%) cases, ASCs were part of the squamous differentiation associated with high-grade urothelial carcinoma (HGUC). In the remaining cases, 8/53 were primary squamous cell carcinoma (SCC) of bladder, 9/53 had HPV infection and 6/53 were reactive/metaplastic. The remaining case was a metastatic SCC from the cervix. 32/53(60%) cases had follow-up biopsies. 13/32 cases showed low-grade squamous intraepithelial lesion (LSIL) and worse, 12 (92%) of which had in situ or invasive cancers in follow-up biopsies.

Conclusions: ASCs in urine cytology are rare, only present in about 1.1% of cases. The most common etiology is in association with HGUCs, followed by squamous cell carcinoma and HPV-related changes. A minority of the cases have reactive inflammatory atypia.

ASCs with cytological atypia is considered a spectrum of abnormal squamous lesions ranging from reactive/metaplastic to HPV-related to squamous cell carcinoma. These lesions should be histologically confirmed with biopsies. The majority of ASCs in the urine are primary lesions, however, rarely, metastatic tumors from non-urinary tract sites may also be encountered, as demonstrated in our study.

404 Comparative Accuracy of Cholangioscopy in Bile Duct Cytology

Sayedamin Mostofizadeh[1], F. Zahra Aly[2], Jacquelyn Knapik[2], Salmaan Jawaid[3], Peter Drew[4], Marino Leon[4], Faisal Mukhtar[4], Robert Klein[5]
[1]Immunology and Lab Medicine, University of Florida, Gainesville, FL, [2]University of Florida College of Medicine, Gainesville, FL, [3]University of Florida Health, Gainesville, FL, [4]University of Florida, Gainesville, FL, [5]University Medical Center, Tucson, AZ

Disclosures: F. Zahra Aly: None; Jacquelyn Knapik: None; Salmaan Jawaid: None; Peter Drew: None; Marino Leon: None; Faisal Mukhtar: None

Background: Biliary duct strictures may be caused by neoplastic or inflammatory processes. Sampling of the bile duct is by brushings and biopsy of the wall of the bile duct. Diagnostic material may be obtained at endoscopic retrograde cholangiopancreatogram (ERCP), by intervention radiology (IR) and, since its introduction in 2015, by single operator cholangioscope (CS).

The specificity of biliary brush cytology in previous studies ranges between 90-100%. The diagnostic sensitivity is modest (35% to 59.8%). The use of CS has been shown to increase the sensitivity to 50-70% likely due to excellent visualization within the bile duct allowing for targeted sampling.

The aims of the present study were to evaluate the overall accuracy of bile duct brushings and compare the accuracy of cytology specimens obtained by ERCP, IR and cholangioscopy.

Design: A retrospective search of the cytology database for bile brushings specimens identified 123 cases (2016 -2018). Pathology slides (cytology, biopsy/resection specimen) and the EPIC information system were reviewed for follow up clinical and imaging data (minimum 8 months). The gold standard for definitive diagnosis of malignancy was surgical biopsy/resection and compelling clinical evidence of malignancy. Definite negative diagnosis relied on lack of clinical/imaging features of malignancy on follow up.

Results: A total of 123 cases were retrieved composing 61 females and 62 males with a median age of 66 years (mean 64.1, range 12-92 years). Cytology brushings were collected during ERCP in 75%, CS in 20%, and IR in 5% of cases. 31% were benign, 66% malignant and in 4 cases the diagnosis could not be determined due to lack of follow up data. There was no difference in diagnostic accuracy of cytology specimens obtained by ERCP, CS or IR. Overall sensitivity for all methods was 73% with specificity of 90% and accuracy of 82%. Notably, in four cases of primary sclerosing cholangitis, the reviewed diagnosis of cytological material was suspicious for malignancy accounting for the 10% false positive rate.

Conclusions: All modalities of sampling were broadly equivalent. Our diagnostic accuracy is similar to that quoted in literature. Patients with primary sclerosing cholangitis are particularly difficult to evaluate cytologically due to presence of single atypical cells and small three dimensional atypical clusters. The use of validated ancillary studies would be particularly impactful in primary sclerosing cholangitis.

405 Fine Needle Aspiration of Solitary Fibrous Tumor (SFT): A 19-Year Retrospective Study of 33 Cases from a Single Institution

Mohamed Mustafa[1], Harvey Cramer[2], Shaoxiong Chen[1]
[1]Indianapolis, IN, [2]Indiana University Health, Indianapolis, IN

Disclosures: Mohamed Mustafa: None; Shaoxiong Chen: None

Background: Solitary fibrous tumor (SFT) is a rare mesenchymal tumor that commonly occurs in the pleura. It shows patternless spindle cell proliferation in a collagenous stroma along with hemangiopericytomatous-like vasculature. SFT diagnosis by FNA can be challenging.

Design: A computerized search in our system was performed for the 19-year period (January 2000 through June 2019) to identify all cytology and surgical pathology cases in which the diagnosis of SFT was rendered or considered in the differential diagnosis. All cytology, surgical pathology reports and related clinical histories were retrospectively reviewed. All of the cytology slides were reviewed, and the final diagnosis as well as the morphological features were re-examined.

Results: A total of 33 SFT cases diagnosed by FNA were found. The age of the patients ranged from 26 to 86 years (mean = 61 years). The male to female ratio was 1.1:1. The size of SFT ranged from 1.6 to 27 cm (mean = 9.5 cm). The FNA diagnoses were classified as follows: SFT (17 cases, 51%), suggestive of SFT (1 case, 3%), spindle cell neoplasm (7 cases, 21%), hypocellular specimen with no atypical cells (4 cases, 12%), non-diagnostic (3 cases, 10%), and other diagnoses (1 case, 3% [case of FNA of lung mass was misdiagnosed as a non-small cell lung carcinoma while the resection showed SFT]). Histologic correlation was available for a total 31 FNA cases (94%). Among 17 cases diagnosed as SFT by FNA, 15 cases (88%) were confirmed histologically while 2 cases did not have histological followup. The one case that was diagnosed as suggestive of SFT on FNA is proved to be atypical thymoma on the resection. Follow-up of the 7 cases diagnosed as spindle cell neoplasm on FNA were all SFTs. Of 7 histologically confirmed cases, there were 4 cases of hypocellular FNA containing only mixed inflammatory cells and 3 FNA cases of nondiagnostic specimens. The predominant morphological pattern noted on the cytology slides is the presence of loose aggregates of spindled cells with oval to tapered

nuclei and scant to moderate amount of cytoplasm. Immunostaining for CD34 on the cell blocks was positive in all SFT cases correctly diagnosed by FNA.

Conclusions: The abovementioned morphological features along with the immunoprofile of CD34 and STAT6 positivity are useful for diagnosing SFT on FNA. All SFT cases on FNA were confirmed histologically. presumably the fibrotic background of SFT results in the failure of FNA to establish correct diagnosis in a significant proportion of cases.

406 Application of the Milan System for Classifying Parotid Gland Neoplasms: A Retrospective 10-Year Study from a Tertiary Medical Center

Vidarshi Muthukumarana[1], Ling Hui[2], Neda Zarrin-Khameh[3], Ya Xu[3]
[1]University of Texas Medical Branch - UTMB, Friendswood, TX, [2]Fort Wayne, IN, [3]Baylor College of Medicine, Houston, TX

Disclosures: Vidarshi Muthukumarana: None; Ling Hui: None; Neda Zarrin-Khameh: None; Ya Xu: None

Background: Milan System for Reporting Salivary Gland Cytopathology (MSRSGC) has changed the approach in diagnosing salivary gland neoplasms and provided a standardize communication platform for better patient care. Our institution has utilized MSRSGC for over a year and we decided to further validate the application of MSRSGC using our data.

Design: We retrospectively reclassified the fine needle aspiration (FNA) diagnosis of parotid gland neoplasms from a 10-year period and calculated the risk of malignancy (ROM) for each MSRSGC category with subsequent surgical resection. We reviewed clinical follow up data of all patients.

Results: There were total 246 cases of parotid FNA from 227 patients with ages ranging from 19 to 85 years old (average 51 years old). Seventeen patients were lost to follow up and 214 patients were alive at the last follow up. ROM for each MSRGC category was calculated in 131 cases based on the surgical pathology diagnosis (Table 1). Forty one of 246 (16.7%) had a confirmed malignant diagnosis (Figure 1). Four were metastasis to salivary gland; 3 of which were categorized as malignant (metastatic liposarcoma, poorly differentiated carcinoma and sebaceous carcinoma) and one (metastatic melanoma) classified as salivary gland neoplasm of uncertain malignant potential (SUMP). The ROM for non-diagnostic (ND) category was 45% and atypia of undetermined significance (AUS) category was 33%; higher than that established in MSRSGC (25% and 20% respectively) (Table 1). Although no false positive cases were identified; there were 5 false negative cases. Scant cellularity and sampling errors were the main reasons for these diagnostic errors. All five patients were alive five years following initial diagnosis.

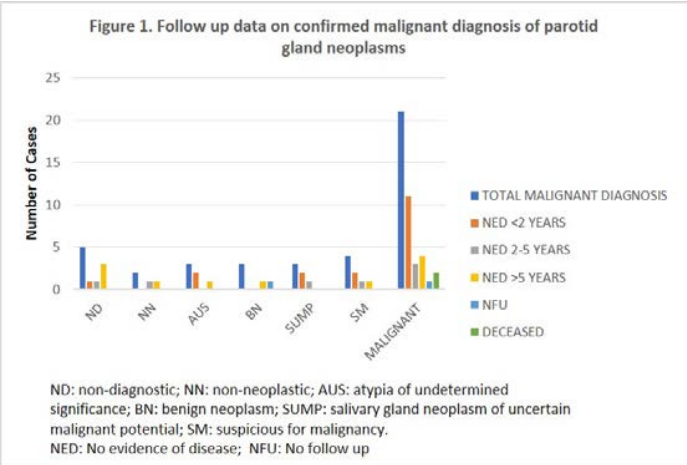
Table 1. Reclassification of parotid gland neoplasms according to Milan system

10-year data

		MSRSGC CATEGORY						
		ND	**NN**	**AUS**	**BN**	**SUMP**	**SM**	**MAL**
FNA Total =246		30	62	21	91	13	6	23
	Benign	6	15	6	56	7	0	0
Histology Total = 131	Malignant	5	2	3	3	3	4	21
	No histology	19	45	12	32	3	2	2
	ROM	45%	11%	33%	5%	30%	100%	100%
ROM by MSRSGC		25%	10%	20%	<5%	35%	60%	90%

ND: non-diagnostic; NN: non-neoplastic; AUS: atypia of undetermined significance; BN: benign neoplasm; SUMP: salivary gland neoplasm of uncertain malignant potential; SM: suspicious for malignancy; MAL: malignant.

Figure 1 - 406



Conclusions: The slightly increased ROM for ND and AUS categories may be an overestimate since 60% of these cases were lacking histology correlation. Although metastasis to parotid are mostly categorized as malignant, rare cases may be classified as SUMP depending on availability of material for work up. The false negative rates can be reduced by obtaining additional material, classifying scant cellularity specimens as ND, rather than non-neoplastic/benign, and using SUMP category for “basaloid” and “oncocytic" lesions. Milan system has proven to be a complementary and effective terminology in salivary gland FNA. Larger prospective studies over time are necessary to improve the ROM and refine each category.

407 Establishing a Standardized p16 Cutoff Value by Immunohistochemistry in Fine Needle Aspirations of Head and Neck Squamous Cell Carcinomas: An Institutional Experience

Rana Naous[1], Vinita Kukkar[2], Ola El-Zammar[3]
[1]UPL LLP, Syracuse, NY, [2]Syracuse, NY, [3]SUNY Upstate Medical University, Syracuse, NY

Disclosures: Rana Naous: None; Vinita Kukkar: None; Ola El-Zammar: None

Background: Fine needle aspiration (FNA) of head and neck (H&N) squamous cell carcinomas (SCC) may in some cases represent the only available diagnostic material. In the absence of a recommended cutoff p16 immunohistochemistry (IHC) value in FNA specimens, determining the HPV status in such cases may pose as a challenge. The aim of this study is to establish a standardized cutoff p16 IHC value in FNAs of H&N SCC.

Design: A retrospective computerized search for FNAs of H&N SCC was carried out at our institution between the period of January 2008 and January 2019. All FNA cases with a corresponding surgical biopsy or excision, in which p16 IHC was performed on the surgical specimen were retrieved. The average percentage of p16 IHC staining in the FNA specimens was determined by 3 different cytopathologists and was correlated with the results of p16 IHC in the surgical specimens. The p16 expression on surgical specimens, using the recommended 70-75% p16 IHC cutoff value, was considered our reference and therefore the determining factor for the p16 IHC status on the corresponding FNA specimens.

Results: A total of 35 cases (22 M and 13 F) were retrieved. The location of the specimens included tongue base, neck, soft palate, tonsils, cervical lymph nodes, nasopharynx and floor of mouth. All cases without p16 IHC performed on the surgical specimen were excluded. 26 surgical cases had a positive p16 IHC using the recommended 70-75% cutoff value, and 9 surgical cases were p16 IHC negative. The latter 9 cases had an average p16 IHC staining of <25% on the corresponding FNA specimens. Of the remaining 26 cases, 23 had a p16 IHC percentage positivity ranging from 25 to 100% on the FNA specimens. The remaining 3 cases had a completely negative p16 IHC (0%) on FNA and positive p16 IHC on the surgical specimen due to the minimal presence of viable tumor cells in the cellblock material. Therefore, a p16 IHC cutoff value of 25% was concluded for FNAs of H&N SCC after correlation with the p16 IHC status on the corresponding surgical specimens.

Conclusions: Based on our findings, the recommended cutoff value for p16 IHC in FNAs of H&N SCC that correlated well with the corresponding surgical specimens in our series was 25%. We recommend using this cutoff as a standard value in determining the p16 IHC status and hence the HPV status on FNA specimens.

408 Should "Suspicious for High-Grade Urothelial Carcinoma" and "Positive for High-Grade Urothelial Carcinoma" Remain Separate Categories?

Luan Nguyen[1], Shikha Bose[2], Rania Bakkar[1]
[1]Cedars-Sinai Medical Center, Los Angeles, CA, [2]Cedars-Sinai Medical Center, West Hollywood, CA

Disclosures: Luan Nguyen: None; Shikha Bose: None; Rania Bakkar: None

Background: The Paris System (TPS) for Reporting Urinary Cytology (1st edition published in December 2015) aims to standardize urinary cytology reporting to help better triage patients for management. Per TPS, the diagnosis of "suspicious for high-grade urothelial carcinoma (SHGUC)" is applied for cases that have few urothelial cells with severe atypia but are quantitatively insufficient for a diagnosis of "high-grade urothelial carcinoma (HGUC)". Criteria for severe atypia of TPS include increased nuclear to cytoplasmic ratio (at least 0.5 - 0.7), moderate to severe nuclear hyperchromasia, and either nuclear membrane irregularities or abnormal clumpy chromatin. How accurate is the diagnosis of SHGUC and HGUC since applying TPS? Would the two categories have similar risk of malignancies (ROM) and therefore could be combined to perhaps improve interobserver variability? To the best of our knowledge, this is the first study to compare the ROM of SHGUC and HGUC categories of TPS in urine cytology cases prospectively categorized by TPS in an effort to determine if the two categories should be condensed.

Design: All urine cases with diagnosis of either SHGUC or HGUC from January 2016 to July 2019 were reviewed by authors to confirm diagnosis. Diagnoses were originally independently rendered by 6 cytopathologists per TPS criteria. Only cases with concurrent biopsy diagnosis (within 6 months after urine specimen collection) were included.

Results: Ninety-eight cases (39 voided and 59 instrumented), 80 of which were obtained from the lower urinary tract, met criteria. Of these 98 cases, 51 had cytology diagnosis of SHGUC and 47 had diagnosis of HGUC. Thirty-five from SHGUC group (69%) and 44 from HGUC group (94%) had biopsy-proven HGUC. A total of 19 cases had biopsies negative for HGUC (16 from SHGUC group and 3 from HGUC group). Eighteen out of the 19 cases with negative biopsies had remote history of urothelial carcinoma and treatment with either intravesical bacillus Calmette-Guerin or mitomycin and 1 had diagnosis of low-grade urothelial carcinoma. The difference in the rate of biopsy-proven HGUC between the SHGUC category and the HGUC category (35/51 compared to 44/47) was statistically significant ($p = 0.001$).

Conclusions: The difference in the ROM between SHGUC and HGUC is statistically significant in our study cohort. Intravesical chemotherapy is frequently observed in negative biopsy cases in both groups. Our preliminary findings suggest that the two TPS categories should remain separate.

409 Nuclear NR4A3 Expression Distinguishes Acinic Cell Carcinoma from its Mimics on Fine Needle Aspiration Biopsy Specimens

Luan Nguyen[1], Fai Chung[1], Mariza De Peralta-Venturina[2], Shikha Bose[2], Bonnie Balzer[1]
[1]Cedars-Sinai Medical Center, Los Angeles, CA, [2]Cedars-Sinai Medical Center, West Hollywood, CA

Disclosures: Luan Nguyen: None; Fai Chung: None; Mariza De Peralta-Venturina: None; Shikha Bose: None; Bonnie Balzer: None

Background: Acinic cell carcinoma of the salivary gland (ACC-SG) is characterized by a recurrent chromosomal rearrangement (t(4;9)(q13;q31)) that upregulates the transcription factor NR4A3. Studies conducted on formalin-fixed paraffin-embedded (FFPE) tissue have found that nuclear expression of a monoclonal antibody NR4A3 (NOR-1) is a sensitive and specific diagnostic marker for ACC-SG (*Haller F, Skalova A et al. Am J Surg Pathol. 2019 Sep; 43(9): 1264-1272*). The diagnosis of ACC-SG is particularly challenging on fine needle aspiration (FNA) as reactive conditions and benign (e.g. Warthin tumor), and malignant neoplasms (e.g. mucoepidermoid carcinoma) enter the differential diagnosis. Recently, successful distinction of ACC-SG from other salivary gland tumors using the NR4A3 monoclonal antibody was reported. We evaluated the performance and utility of NR4A3 for separating ACC-SG from its mimics on cytology cell block specimens.

Design: Ten salivary gland FNA specimens with adequate cell block material were retrieved from our database from a 13-year period (2006 to 2018). These included 5 cases of ACC-SG, 2 cases with abundant benign acinic cells, 2 case of Warthin's tumor, and 1 case of mucoepidermoid carcinoma. The diagnosis of all cases was confirmed on subsequent surgical excision specimens. An automated immunohistochemistry system (Bond-III, Leica) was used for the detection of NR4A3, using a commercially available antibody (sc-393902 [H-7], Santa Cruz Biotechnology Inc.). Optimization of the antibody on the cell blocks was successfully completed by increasing the titer from 1:100 (suggested titer per Haller et. al.) to 1:30. The immunoreactivity for NR4A3 was compared between the cell blocks and their corresponding surgical pathology specimens.

Results: Distinct nuclear reactivity for NR4A3 was observed in all 5 cases of ACC-SG (4 out of 5 with 2-3+ diffuse nuclear positivity). Expression of NR4A3 was absent in all non-ACC-SG cases. Comparison to the corresponding FFPE surgical cases was made initially at the 1:100 titer level; however, the cell block positive staining was very faint on a few cytology cell blocks. After we increased the titer to 1:30 titer, the immunoreactivity improved significantly on the cytology cell blocks.

Conclusions: Fine needle aspirates of salivary gland tumors with adequate cell block material in which ACC-SG is a diagnostic consideration provide an early and valuable opportunity to separate ACC-SG from its mimics using NR4A3 immunohistochemistry.

410 Next-Generation Sequencing of Cell-Free DNA Extracted from Pleural Effusion Supernatant: Applications and Challenges

Ami Patel[1], Lucelina Rosado[2], Lin Cong[2], Susan Alperstein[3], Hanna Rennert[4], Momin Siddiqui[1], Jonas Heymann[4]
[1]New York-Presbyterian/Weill Cornell Medical Center, New York, NY, [2]New York-Presbyterian/Weill Cornell Medicine, New York, NY, [3]Weill Cornell Medicine, Fishkill, NY, [4]Weill Cornell Medicine, New York, NY

Disclosures: Ami Patel: None; Lucelina Rosado: None; Lin Cong: None; Susan Alperstein: None; Hanna Rennert: None; Momin Siddiqui: None; Jonas Heymann: None

Background: Molecular profiling requires high quality tumor DNA, such as cell-free DNA (cfDNA) extracted from plasma or other biosources, especially when only a small biopsy is available or when DNA extracted from conventional formalin-fixed, paraffin-embedded (FFPE) tissue is inadequate. cfDNA extracted from post-centrifugation malignant pleural effusion supernatant (SN) is characterized herein.

Design: Fresh pleural effusion specimens of metastatic lung adenocarcinoma were collected prospectively. After ThinPrep (TP) and cell block (CB) preparation, DNA was extracted from SN with the MagMAX Cell Free DNA Isolation Kit (ThermoFisher) and analyzed with the High Sensitivity DNA Assay (Agilent). Next-generation sequencing (NGS) libraries were prepared and analyzed with the Oncomine Lung cfDNA Assay (ThermoFisher). Results were compared with corresponding FFPE samples. A cytopathologist and a cytotechnologist evaluated tumor cell cohesion and necrosis and tumor (TC) and overall cellularity (OC) on TP and CB slides using quantitative and qualitative metrics.

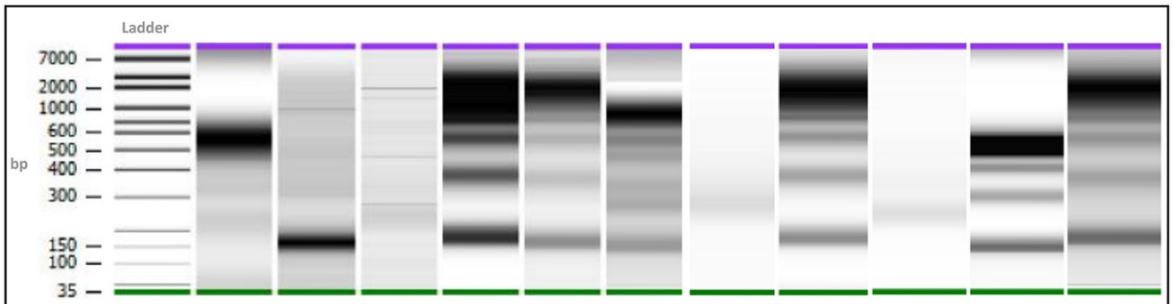
Results: 11 specimens were collected from 9 patients; TP slides were available for 10 specimens from 8 patients. Specimen volumes, overall cellularity (OC), and tumor cellularity (TC) are detailed in the Table. OC and TC on CB sections correlated well with corresponding TP slides.

Med (med) extracted DNA yield and library concentration are detailed in the Table. Med DNA yield for specimens of low or moderate TP OC was higher than for specimens of high TP OC (53 vs. 7 ng/µL). Med read coverage ranged from 208-127,752x; 8 samples had >10,000x coverage. Extracted DNA consisted of both cell-free and cellular fractions(Figure).

Driver mutations were identified in 4/9 patients (*KRAS*=2, *EGFR*=2, Table) and in a sample with no tumor cells on TP (<1% TC on corresponding CB). *TP53* mutation was also identified in 2 patients. NGS of molecular drivers was concordant in 8/10 samples with an adequate FFPE control sample. No correlation was identified between sample volume or OC, quality or quantity of extracted DNA, or mutation detection.

Total Specimens (n=11)	
Volume (mL)	
Median	170
Range	35-1,400
Gross Appearance	
Bloody	7
Cloudy/Clear	4
Extracted DNA (ng/µL)	
Median	36.0
Range	0.1-58.0
Library Concentration (pM)	
Median	458
Range	90-3,118
Identified Mutations	
KRAS	2
EGFR Only	1
EGFR and *TP53*	1
TP53 Only	1
Mutant Allelic Fraction (%)	
Median	23.3
Range	0.6-44.3
ThinPrep Slides (available n=10)	
Overall Cellularity	
Median	24,000
Range	<1,000-110,000
Tumor Cellularity (%)	
Median	15
Range	<5-95
Predominant Pattern	
Single Tumor Cells	6
Tumor Cell Clusters	4

Figure 1 - 410



Sample	1	2	3	4	5	6	7	8	9	10	11
Overall Cellularity (ThinPrep)	>100,000	36,000	<1,000	5,000	N/a	4,000	>100,000	90,000	13,000	90,000	<1,000
Tumor Percent (%)	<5%	40%	>95%	15%	N/a	15%	15%	10%	10%	25%	>95%

Conclusions: Mutations are identifiable in high quality DNA extracted from SN, including those for which TC in FFPE is inadequate for NGS, as well as those with low volume or OC. Here, NGS was concordant for driver mutations in a majority (80%) of samples with an adequate FFPE control. Despite challenges, including variation in sample quantity and quality of extracted DNA, SN represents a source of DNA upon which NGS is feasible.

411 Adrenal Gland Fine Needle Aspiration: A Multi-Institution Retrospective Analysis of 139 Cases

Kimberly Point du Jour[1], Yazeed Alwelaie[1], Arlixer Coleman[2], Tadros Talaat[3], Michelle Reid[1]
[1]Emory University Hospital, Atlanta, GA, [2]Grady Hospital, Atlanta, GA, [3]Emory University, Atlanta, GA

Disclosures: Kimberly Point du Jour: None; Yazeed Alwelaie: None; Arlixer Coleman: None; Tadros Talaat: None; Michelle Reid: None

Background: Adrenal gland lesions span a wide range of entities from benign and malignant primary neoplasms to metastatic tumors. Fine needle aspiration (FNA) provides a minimally invasive diagnostic tool to stage patients with known malignancy and procure material for molecular testing. The goal of this study was to characterize the clinicopathologic features of FNA of the adrenal gland from two large academic institutions.

Design: Adrenal gland FNAs were identified by query of the electronic medical record from 2002- 2019. Clinical and pathological information was collated and correlated with the corresponding surgical diagnosis when available.

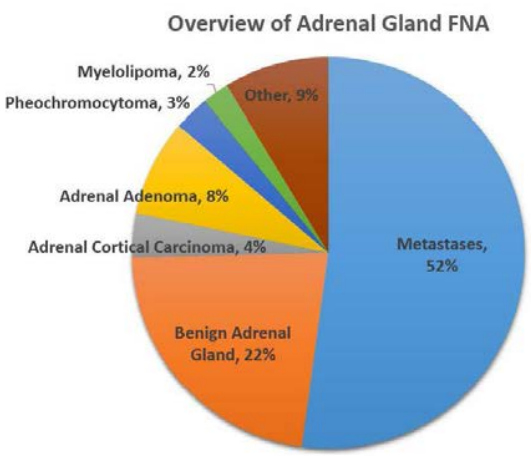
Results: Of 139 cases, there were 72 males and 67 females (M:F 1:1) of median age 65 years (range 21-90) with median mass size 3.2 cm (range 1.0-22.9), 64 (46%) of which were on the right. A majority (91%, n=127) of FNAs were CT-guided, followed by endoscopic ultrasound (7%, n=10) and MRI (2%, n=1). FNA diagnoses included 12 (9%) non-diagnostic FNAs, 32 (23%) negative for malignant cells, 16 (12%) neoplasms, 2 (1%) atypical, 1 (1%) suspicious and 76 (54%) positive for malignancy.

Over half (52%, n=72) of the adrenal masses were metastatic tumors, 23 (17%) were primary adrenal neoplasms, 31 (22%) were normal adrenal and one case of disseminated histoplasmosis. Metastatic tumors were most frequently carcinoma (53, 74%), followed by melanoma (11, 15%), lymphoma (4, 6%) and sarcoma (4, 6%). Site of origin of metastatic carcinomas was most frequently lung (21, 29%) followed by genitourinary (12, 17%) and hepatobiliary/gastrointestinal tract (11, 15%) [Fig. 1]. Primary adrenal neoplasms included 11 (48%) adenomas, 5 (22%) adrenal cortical carcinomas, 4 (17%) pheochromocytomas, and 3 (13%) myelolipomas. One case diagnosed as an adrenal neoplasm proved to be metastatic carcinoma on resection. Of the 8 resected non-diagnostic or negative cases, 2 proved to be metastases (melanoma; renal cell carcinoma) and 4 were benign adrenal neoplasms (3 adenomas; 1 myelolipoma) [Table]. Thirty-two patients with metastases died at median time to death of 8 months.

Metastatic Tumors to the Adrenal Gland					
Primary site	n	%	Histologic type	n	%
Carcinoma	53	74	Adenocarcinoma	24	45
Lung	21	29	Squamous cell carcinoma	5	9
Genitourinary	12	17	Small cell carcinoma	3	6
GI/hepatobiliary	11	15	Poorly differentiated carcinoma	2	4
Larynx	3	4	Renal cell carcinoma	10	19
Breast	2	3	Hepatocellular carcinoma	7	13
Cervix	1	1	Urothelial carcinoma	2	4
Oropharynx	1	1			
unknown	2	3	Total	53	100
Melanoma	11	15			
Lymphoma	4	6			
Sarcoma	4	6			
Total	72	100			
Primary Adrenal Neoplasms					
	n	%			
Adrenal neoplasms	23	100			
Adrenal adenoma	11	48			
Adrenal cortical carcinoma	5	22			
Pheochromocytoma	4	17			
Myelolipoma	3	13			
	12	9			
Negative and Non-Diagnositic FNA with Resection Diagnosis					
	n			n	
Non-diagnostic	12		Negative	32	
Resected	5		Resected	3	
Resection diagnoses			*Resection diagnosis*		
Adrenal neoplasm	2		Adrenal adenoma	1	
Myelolilpoma	1		Histoplasmosis	1	
Non-neoplastic inflammatory lesion	1		Metastatic clear cell renal cell carcinoma	1	
Metastatic melanoma	1				

Figure 1 - 411

Figure 1.



Conclusions: High adequacy and low false negative rates are achieved with adrenal gland FNA. While most adrenal masses represent metastatic carcinoma from lung, other primary sites, including those below the diaphragm, should also be considered in the diagnostic differential. In patients with metastatic carcinoma, the presence of adrenal metastasis represents a poor prognostic sign with median survival of 8 months.

412 Success Rate of Percutaneous Needle Core Biopsies in Diagnosing Renal Mass Lesions and their Concordance with Rapid On-Site Evaluations Using Touch Preparations

Joaquin Ponce-Zepeda[1], Behdokht Nowroozizadeh[2], Di Lu[1], May Myint[1], Min Han[1]
[1]University of California Irvine, Orange, CA, [2]Cerritos, CA

Disclosures: Joaquin Ponce-Zepeda: None; Behdokht Nowroozizadeh: None; Di Lu: None; May Myint: None; Min Han: None

Background: Small renal mass biopsies, including needle core biopsy (NCB) and fine needle aspiration (FNA) have not been commonly performed due to the perceived low sensitivity until recent years. Rapid on-site evaluation (ROSE) has the potential to improve the yield of small renal biopsies, however, it can be challenging due to the diverse morphology of various types of renal tumors and even native kidney cells. We sought to study the success rate of NCB in diagnosing renal mass lesions and the concordance of ROSE using touch preparations (TPs) with final diagnosis on CNB.

Design: Eighty-seven percutaneous CNBs of renal masses with concurrent TPs for ROSE were retrieved in our institutional data base. ROSE findings were correlated with the final diagnosis on NCB. The number of needle cores acquired were recorded.

Results: NCB led to the diagnoses of malignant or benign neoplasms in 83.9% (73/87) cases, suspicious for malignancy in 2.3% (2/87), atypical cells in 2.3% (2/87) and benign in 11.5% (10/87) cases. ROSE findings were divided into the following categories: inadequate (n=5), negative (n=10), atypical (n=26), neoplasm (n=3), suspicious (n=1), malignant (n=13) and adequate with no interpretation (n=31). ROSE findings were concordant with the final reports in 88.5% (77/87) cases (table 1). Discrepancies between ROSE and final diagnoses included 1) preparation error/inadequate cells on TPs but tumor cells present on NCBs in 4 cases, 2) under-calling/interpreted as negative during ROSE but final CNB showed malignancy/neoplasm in 3 cases, and 3) minor over-calling/ reported as atypical cells during ROSE but final CNB was negative in 3 case. Subsequent kidney resections were found in 39% (34/87) of the biopsied cases, all of which showed renal cell carcinomas, concordant with NCB results in all except for 2 cases. Tumor was not present in the CNBs or TPs in these two cases, representing true sampling error. The number of needle cores acquired ranged between 2-7 (mean 2.8) for the neoplastic/malignant cases, 2-7 (mean 4.2) for the negative cases, 4-5 (mean 4.5) for the suspicious cases and 5 for the two atypical cases.

Table 1. Concordance and Discordance between ROSE Interpretations and CNB final Diagnosis			
ROSE Interpretation (total =87)	N	Concordant CNB Findings (n=77)	Discordant CNB Findings (n=10)
Inadequate	5	Benign adipose tissue (1)	CCRCC (2) ChrRCC (1) RCC, not further classified (1)
Negative	10	Benign (6) Atypical (1)	CCRCC (1) PRCC (1) AML(1)
Atypical	24	Atypical (1) Suspicious (1) Malignant (19) • CCRCC (10) • PRCC (2) • ChrRCC (2) • ACDRCC (1) • RCC, not further classified(4)	Benign kidney (3)
Neoplasm	3	Oncocytoma (1) PRCC (1) ChrRCC (1)	None
Suspicious	1	CCRCC (1)	None
Malignant	13	CCRCC (7) PRCC (1) ChrRCC (1) Sarcomatoid RCC (1) CCPRCC (1) RCC, not further classified (2)	None
Adequate, no interpretation	31	CCRCC (14) PRCC (3) ChrRCC (4) CCPRCC (1) Urothelial ca (1) Oncocytoma (2) RCC, not further classified (2) Suspicious for RCC (1) Lymphoma (1) AML (2)	None
Total		CCRCC (32) PRCC (7) ChrRCC (8) CCPRCC (2) ACDRCC (1) Sarcomatoid RCC (1) RCC, not further classified (8) Suspicious (2) Urothelial carcinoma (1) Lymphoma (1) Oncocytoma (3) AML (2) Atypical (2) Benign (7)	CCRCC (3) PRCC (1) ChrRCC (1) RCC, not further classified (1) AML (1) Benign kidney (3)

Note: CCRCC – clear cell renal cell carcinoma, PRCC – papillary renal cell neoplasm, ChrRCC – chromophobe renal cell carcinoma, CCPRCC – clear cell papillary renal cell carcinoma, ACDRCC - acquired cystic disease associated RCC, AML – angiomyolipoma

Conclusions: Percutaneous CNB has a high success rate in diagnosing renal mass lesions. TP has a high concordance rate (88.5%) with the final NCB results, providing valuable confirmation during on-site evaluations. The number of needle cores acquired during the biopsy does not seem to correlate with the likelihood of finding malignancy/neoplasm.

413 Effusion Fluids: Utilization of Next-Generation Sequencing

Liza Quintana[1], Yubo Wu[1], Paul VanderLaan[1], Athena Chen[1]
[1]*Beth Israel Deaconess Medical Center, Boston, MA*

Disclosures: Liza Quintana: None; Yubo Wu: None; Paul VanderLaan: *Consultant*, Foundation Medicine; Athena Chen: None

Background: Molecular testing of pathology specimens is becoming increasingly important for providing diagnostic, prognostic, and predictive information. Next-generation sequencing (NGS) may be employed in the metastatic setting in an attempt to identify targetable mutation(s) or allow patients the opportunity to join clinical trials with specific eligibility requirements. We reviewed our institutional experience with NGS on metastases to effusion fluids to evaluate the utility of this specimen type as a substrate.

Design: Effusion fluid cell block (CB) specimens sent to Foundation Medicine for NGS between 1/2017-8/2019 were retrospectively identified and specimen characteristics were recorded. CB at our institution are formalin-fixed paraffin-embedded.

Results: 1,051 specimens were sent from our institution to Foundation Medicine for NGS during the study period. Of those with reports available, 32 from 31 patients were effusion fluid CBs. The fluid metastases included 6 breast carcinomas, 1 cervical adenosquamous carcinoma, 1 esophageal adenocarcinoma, 18 lung adenocarcinomas, 1 Mullerian adenocarcinoma, and 5 pancreatic ductal adenocarcinomas (PDAC). 24/32 (75%) had adequate tumor cellularity for NGS: 4 breast, 1 cervical, 1 esophageal, 15 lung, 1 Mullerian, and 2 PDAC. The median fluid volume was 800 mL (range 25-1200 mL). Of the 24 sufficient specimens, the median fluid volume was 800 mL and of the 8 specimens deemed insufficient, the median volume was 800 mL (p=0.93, Wilcoxon rank-sum test). One insufficient specimen was from a patient with breast carcinoma who had a separate specimen with sufficient tumor submitted and 5 had scant tumor cellularity noted on CB. All 24 specimens which underwent NGS had high tumor cellularity present in the CB and had genomic alterations identified. 16/24 (67%) had targetable genomic mutations.

Conclusions: Patients with metastatic malignancies may have limited treatment options; thus, NGS may be an important adjunct in their clinical management, identifying mutations with targeted therapies or that allow patients enter clinical trials. As such, we reviewed our experience with NGS on effusion samples. While these specimens were infrequently sent for NGS, we find that if sufficient tumor is present in the CB, they are reliable substrates for testing. Cellularity and sufficiency for testing did not appear to be functions of volume.

414 Utilization of Cytology Smears for NGS-Based RNA Fusion Testing Improves Adequacy Rates

Nisha Ramani[1], Mark Routbort[1], Keyur Patel[1], Russell Broaddus[2], Hui Chen[1], Asif Rashid[1], Alexander Lazar[1], L. Jeffrey Medeiros[1], Jawad Manekia[1], Hyvan Dang[1], Rajyalakshmi Luthra[1], John Stewart[1], Sinchita Roy-Chowdhuri[1]
[1]*The University of Texas MD Anderson Cancer Center, Houston, TX,* [2]*University of North Carolina School of Medicine, Chapel Hill, NC*

Disclosures: Nisha Ramani: None; Mark Routbort: None; Keyur Patel: None; Russell Broaddus: None; Hui Chen: None; Asif Rashid: None; Alexander Lazar: None; L. Jeffrey Medeiros: None; Jawad Manekia: None; Hyvan Dang: None; Rajyalakshmi Luthra: None; John Stewart: None; Sinchita Roy-Chowdhuri: None

Background: Cytopathologists are routinely confronted with molecular test requests on small specimens obtained by fine-needle aspiration (FNA). RNA-based next-generation sequencing (NGS) assays are increasingly being used for comprehensive molecular profiling due to the expanded use of targeted therapeutics in solid tumors. The cell block (CB) is the most commonly used cytology specimen for molecular testing; however, insufficient cellularity and/or RNA quality may affect the performance of these assays. The objective of this study was to assess the potential value of Diff Quik (DQ) and Papanicolaou (PAP) stained smears in a cohort of cases where the CB specimens were inadequate for RNA fusion testing.

Design: Retrospective review was performed for all the submitted requisitions for targeted RNA-based NGS on cytology specimens from January 1 through April 30, 2018. For RNA-based NGS assays, performed on the Ion Torrent platform (ThermoFisher), adequacy criteria of ≥300 cells and ≥20% tumor in CB tissue sections were used. Cytology smears were used when the CB specimens were inadequate for RNA fusion testing. RNA was extracted using the AllPrep kit on a QIAcube liquid handling platform (Qiagen). cDNA prepared from extracted RNA was combined with targeted amplicon-based NGS to amplify a set of targeted fusion sequences corresponding to clinically relevant known inter- and intragenic fusions in 51 genes. Sequences were aligned against a synthetic fusion genome and fusions were identified by coverage analysis.

Results: The study included 80 patients with a median age of 66 (range, 44-88) years and a male to female ratio of 0.8:1. The patient demographics and pathologic characteristics are summarized in Table 1. CB specimens were available and adequate for RNA-based NGS testing in 33/80 (41.2%) cases. With the inclusion of DQ and PAP smears, the adequacy rate of these specimens was 72/80 (90%). The overall failure rate of RNA fusion testing was 10% (8 cases), usually due to suboptimal RNA quality or quantity. Of the 72 cases successfully sequenced, 68 (94.4%) had no detectable fusions and 4 (5.6%) had fusions detected (Table 1).

Table 1. Next-Generation Sequencing-based RNA Fusion Assays for Clinically Relevant Genes in Cytology Samples

Patient Demographics
• Male n=36 • Female n=44 • Age (median): 66 (range, 44-88) years
Site
• Lymph node n=43 • Lung n=14 • Pleura n=6 • Bone and soft tissue n=5 • Thyroid gland n=5 • Liver n=2 • Adrenal gland n=2 • Stomach n=1 • Duodenum n=1 • Parotid gland=1
Diagnosis
• Adenocarcinoma n=44 • Poorly differentiated carcinoma n=7 • Carcinoma (not otherwise specified) n=7 • Papillary thyroid carcinoma n=4 • Non-small cell carcinoma n=4 • Squamous cell carcinoma n=2 • Others (sarcoma, melanoma, gastrointestinal stromal tumor) n=12
Cytology Samples for NGS n=80
• In-house specimens n=73 • Outside specimens n=7
RNA yield • Mean, 0.07 µg/µL • Median, 0.04 (range, 0.002-0.4) µg/µL
Adequacy Rate • Only CBs n=33 (41.2%) • Including smears n=72 (90%) • Only smears: n=27 (33.8%) • Smears and CB combined n= 12 (15%)
RNA NGS Fusion Assay Results
Failed n=8 (10%) • Outside case n=0 • Necrosis n=1 (12.5%) • Only CB n=2 (25%) • Only smears n=6 (75%)
No fusion detected n=68 (85%)*
Fusion detected n=4 (5%) • 1 NCOA4-RET fusion, no confirmation by FISH • 1 NCOA4-RET fusion, but negative by FISH • 2 MET-MET fusions indicating MET Exon 14 Skipping

* One fusion negative case showed deletion of 3' RET by FISH assay suggesting RET rearrangement

Abbreviations: CB, cell block; FISH, fluorescence in situ hybridization; NGS, next-generation sequencing

Conclusions: The success of RNA-based NGS fusion testing is multifactorial and frequently depends on the quality and quantity of RNA extracted. The utilization of DQ and PAP stained smears for RNA fusion testing significantly improves the adequacy of cytologic samples for molecular testing.

415 Accuracy of Grading of Well-Differentiated Pancreatic Neuroendocrine Tumors (PanNET) on Fine Needle Aspiration (FNA): A Multi-Institutional Study Using One Counting Method

Michelle Reid[1], Yin Xiong[2], Kim HooKim[3], Nirag Jhala[4], Emilio Madrigal[5], Martha Pitman[6], Muhammad Idrees[7], Barbara Centeno[2]
[1]Emory University Hospital, Atlanta, GA, [2]Moffitt Cancer Center, Tampa, FL, [3]Thomas Jefferson University, Philadelphia, PA, [4]Temple University Hospital, Philadelphia, PA, [5]Boston, MA, [6]Massachusetts General Hospital, Harvard Medical School, Boston, MA, [7]Indiana University School of Medicine, Indianapolis, IN

Disclosures: Michelle Reid: None; Yin Xiong: None; Kim HooKim: None; Nirag Jhala: None; Emilio Madrigal: None; Martha Pitman: None; Muhammad Idrees: None; Barbara Centeno: None

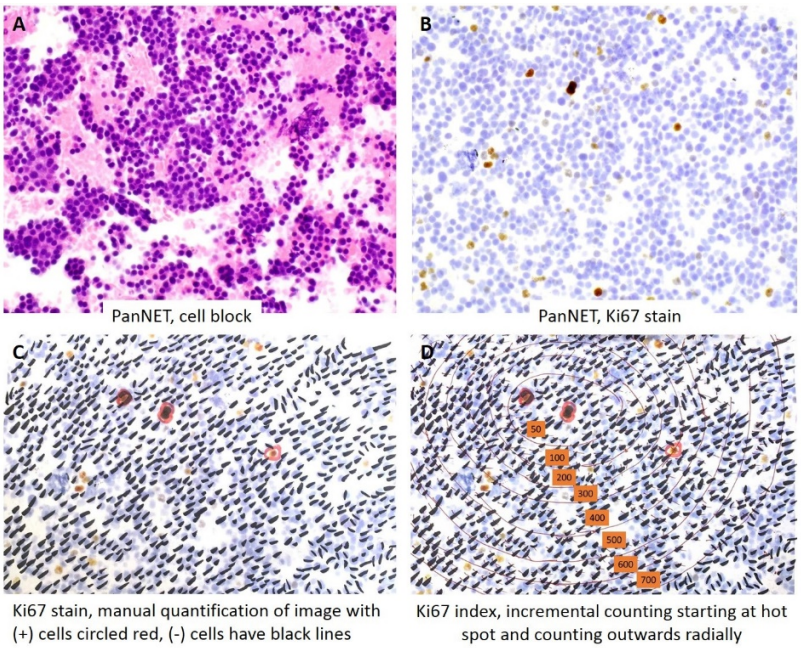
Background: PanNETs are graded based on ki-67 proliferation index (KI), as defined by the WHO 2017. KI in resected PanNETs is established by averaging 3 hot spots of minimum 500 cells. Accuracy of grading PanNETs on endoscopic ultrasound-guided FNA has not been standardized. We sought to determine the reproducibility of grading PanNETs on FNA, and to determine the minimum number of tumor cells required for accuracy concordance between cell block (CB)-generated KI versus that of corresponding resections.

Design: Included in this multi(6)-institutional study were patients with resected PanNET who had preoperative FNA with available ki-67 stained CBs. Hot spots were identified by scanning ki-67 slides at 10X. Up to 3 hot spots/slide were captured at 20X, and the still image used for manual KI quantification, KI which was done by marking positive and negative tumor cells. On FNAs KI was calculated in 3 hot spots using 6 incremental cell counts: 50, 100, 200, 300, 400 and 500 cells maximum. For each hot spot incremental calculation was based on starting the count in the inner circle of the image and expanding outward radially. [Figure] For cases with >1 hot spot, the average of the hotspots was used for analysis. For statistical analysis, mean values were calculated for each FNA cell count. Each averaged grade per incremental cell count was compared to histologic grade (also manually quantified). Chi-square analyses were performed to evaluate most accurate cell count.

Results: KI was calculated on 106 paired FNAs and resections (77 grade 1 (G1), 27 G2 and 2 G3) and there was overall 78% concordance between paired samples using a 500 cell count. Table 1 demonstrates number of cases per cell count and rate of correct grading overall and by grade. The best concordance was with at least 300 tumor cells counted (p=0.0119). Using 300 cells, 85% of G1 tumors were correctly classified on FNA and 54% of G2 were correctly classified. Although counting 500 cells improved G1 accuracy (90%), it did not change accuracy for G2 (52%). All G3 tumors were under-graded as G2 on all counts.

Ki67 index/ cells counted	# Total cases	# Correct overall (%)	# Incorrect overall (%)	Grade 1 #correct/total (%)	Grade 2 #correct/total (%)	Grade 3 #correct/total (%)
Ki67 FNA 50	106	51 (48)	55 (52)	32/77 (40)	18/27 (67)	0/2 (0)
Ki67 FNA 100	102	59 (58)	43 (42)	43/74 (58)	16/26 (62)	0/2 (0)
Ki67 FNA 200	101	69 (68)	32 (32)	53/73 (73)	9/26 (56)	0/2 (0)
Ki67 FNA 300	96	72 (75)	23 (25)	58/68 (85)	14/26 (54)	0/2 (0)
Ki67 FNA 400	91	67 (74)	24 (26)	55/65 (85)	12/24 (50)	0/2 (0)
Ki67 FNA 500	86	67 (78)	19 (22)	55/61 (90)	12/23 (52)	0/2 (0)

Figure 1 - 415



Conclusions: This study shows that using hot spot selection, a minimum 300 cells are required on CB to obtain the best reproducibility on G1 resections. G2 PanNETs are consistently underestimated on all cell counts including 500 cell count. A larger cohort currently being examined by our group would likely reinforce and provide more clarity to these observations.

416 Diagnostic Utility of p16 in Cytology Specimens

Efrain Ribeiro[1], Zahra Maleki[1]
[1]Johns Hopkins Hospital, Baltimore, MD

Disclosures: Efrain Ribeiro: None; Zahra Maleki: None

Background: HPV-related oropharyngeal squamous cell carcinomas (OPSCC) continue to rise in incidence. A neck mass is the initial presentation of HPV-related OPSCC in up to 50% of the cases while the primary site remains unknown in 10% of cases. Utility of p16 immunostaining as a surrogate marker for HPV remains controversial, as p16 is expressed in a variety of other conditions. The aim of this study is to explore diagnostic utility of p16 on cytology specimens.

Design: After IRB approval, the electronic data of a large academic institution was retrospectively (2014 -2018) searched for cytology cases accompanied by p16. The patients' sex, body site and diagnoses were recorded. Cases were categorized based on their region to 1) Head/Neck, 2) Thorax 3) Genitourinary, and 4) Gastrointestinal. p16 staining was quantified as negative, patchy, or diffuse. HPV testing was recorded where available.

Results: 398 cases were identified, 259 men (26–99 years, mean = 61.7 years) and 139 women (25–95 years, mean = 60.7 years). There was a significant gap in head/neck (160 M vs. 44 F) and abdominal cases (1 M vs. 30 F) where p16 IHC was used (Fig. 1). The intensity of p16 staining for all diagnoses and sites was shown in Table 1 and Figure 2. Diffuse staining is seen in a majority of squamous cell carcinomas, small cell carcinomas, and gynecologic serous carcinomas. p16 expression was mostly patchy or negative in adenocarcinomas, various carcinomas, spindle cell neoplasms, atypical cellular proliferations, and benign conditions. Concurrent HPV testing was done on 217 cases. In 138 cases with diffuse staining, 127 were HPV(+) and 11 HPV(-). In 20 cases with patchy staining, 6 were HPV(+) and 14 were HPV(-). In 59 cases with negative staining, 3 were HPV(+) and 56 were HPV(-).

	Diffuse	Patchy	Negative
Squamous Cell Carcinoma	168	16	62
Carcinoma (unspecified)	11	8	9
Small cell carcinoma	7	0	1
Adenocarcinoma	11	7	19
Sarcoma	1	0	2
Epithelioid Carcinoma	1	0	2
Serous Carcinoma	10	2	2
Anaplastic Thyroid Carcinoma	1	0	0
Basaloid Epithelial Carcinoma	0	0	1
Mucoepidermoid Carcinoma	0	0	1
Neuroendocrine Tumor	0	0	1
Spindle Cell Neoplasm	0	0	1
Atypical cells	0	2	8
Benign	0	1	9

Figure 1 - 416

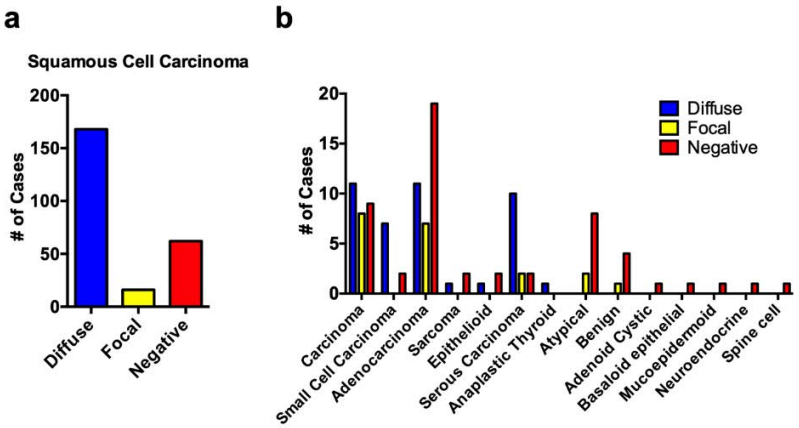
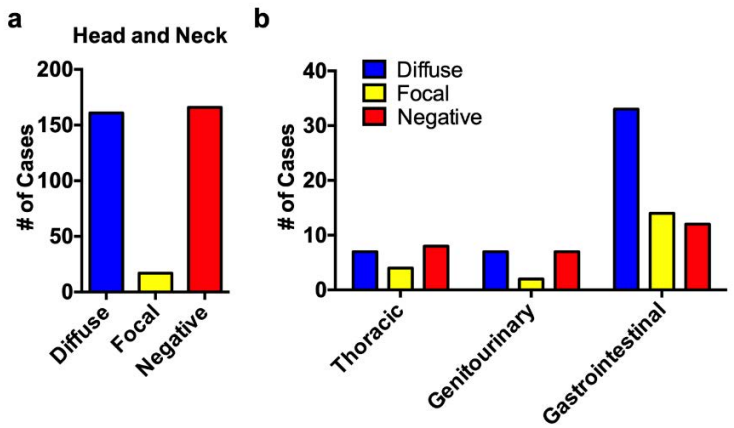


Figure 2 - 416



Conclusions: Our study suggests that p16 IHC is useful in diagnosis of cytology specimens and investigating the primary site in metastatic lesions. Moreover, intensity of P16 carries a diagnostic value. Diffuse p16 staining is strongly associated with HPV (+) status across the body and it is most useful in diagnosis of HPV-related oropharyngeal carcinomas. However, diffuse p16 expression in other neoplasms and organs may possess a diagnostic pitfall. Focal/patchy p16 expression is less common in HPV(+) carcinomas and is seen in other benign and malignant conditions. In summary, cytomorphologic correlation with intensity of p16, history and other ancillary studies such as p40 immunostaining and HPV testing may improve diagnostic accuracy.

417 Application of Milan System for Reporting Salivary Gland Cytology: A Retrospective Cyto-Histological Correlation and Risk Stratification Study in a Tertiary Care Center

Juan Rong[1], Farnaz Hasteh[1]
[1]*University of California San Diego, La Jolla, CA*

Disclosures: Juan Rong: None; Farnaz Hasteh: None

Background: Fine needle aspiration (FNA) has become widely accepted as an initial diagnostic approach in the management of salivary gland lesions. Our department has recently implemented the Milan System for reporting salivary gland cytopathology with the goal of using a defined set of diagnostic categories for improved clinical management. The aim of this study is to apply the Milan System diagnostic categories to our older system and evaluate the frequency, risk of malignancy, and percent discrepancy in comparison to the obtained surgical outcomes.

Design: A retrospective study of salivary gland FNAs in our pathology database from 2010 to 2019 was conducted. The Milan System diagnostic categories were obtained from or assigned based on the original pathology reports. Follow-up histological diagnoses, if available, were recorded as gold standard diagnoses. The frequency and risk of malignancy for each diagnostic category were calculated. In addition, we assessed the percent discrepancy for diagnostic categories II, IVA and VI.

Results: A total of 313 salivary gland FNAs were retrieved: 171(55%) males and 142 (45%) females with a mean age of 60 years (range: 19-96 years). 89% FNAs were collected from parotid gland, and the rest from submandibular gland (5%), neck (5%) and parapharyngeal space (1%). The frequency, risk of malignancy and percent discrepancy of the Milan System diagnostic categories were calculated and summarized in Table 1. Overall, 11% cases were classified as category I (non-diagnostic), 25% as II (non-neoplastic), 9% as III (atypia of undetermined significance), 31% for IVA (benign neoplasm), 8% for IVB (salivary gland neoplasm of uncertain malignant potential), 4% for V (suspicious for malignancy) and 12% for VI (malignant). 160 (51%) FNAs had follow-up histological diagnoses. Based on the final histological diagnoses, risk of malignancy was 6% for category I, 6% for II, 29% for III, 2% for IVA, 41% for IVB, 91% for V, and 95% for VI. Percent discrepancy was 24% for category II, 2% for IVA and 5% for VI.

Milan system	# FNAs	# histology	# malignant	# discrepancy
Diagnostic category	(overall %)	follow-up (%)	(ROM, %)	(% discrepancy)
I. Non-diagnostic	35 (11)	17 (49)	1 (6)	
II. Non-neoplastic	79 (25)	17 (22)	1 (6)	4 (24)
III. AUS	29 (9)	17 (59)	5 (29)	
IVA. Benign neoplasm	97 (31)	61 (63)	1 (2)	1 (2)
IVB. SUMP	25 (8)	17 (68)	7 (41)	
V. SM	11 (4)	11 (100)	10 (91)	
VI. Malignant	37 (12)	20 (54)	19 (95)	1 (5)
AUS: atypia of undetermined significance; SUMP: salivary gland neoplasm of uncertain malignant potential; SM: suspicious for malignancy; ROM: risk of malignancy				

Conclusions: Based on a single institution's experience, the most frequently encountered diagnostic category of the Milan System is benign neoplasm. The reported frequency and risk of malignancy herein are in concordance with the recently published data with frequencies for AUS and SUMP less than 10%, and ROM 29% for AUS and 41% for SUMP. In our opinion, poorly sampled lesions and cytomorphologic overlaps are the two major factors resulting in a diagnostic discrepancy.

418 Cumulative Risk of HSIL During a Long-Term Follow-up for Women with Pap-/HPV+ Baseline Testing Results in a Cervical Cancer Screening Population and a Cancer Surveillance Population

Suvra Roy[1], Abha Khanna[1], Ming Guo[1]
[1]*The University of Texas MD Anderson Cancer Center, Houston, TX*

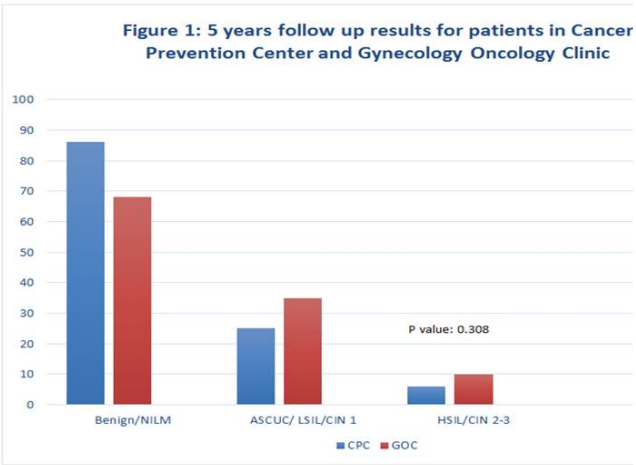
Disclosures: Suvra Roy: None; Abha Khanna: None; Ming Guo: None

Background: The aim of the study was to evaluate the 5-years and longer time risk of high-grade cervical intraepithelial lesion (HSIL) in women with Pap-negative/HPV-positive co-testing results in a cervical cancer screening population and a cancer surveillance patient population in our institution.

Design: We retrospectively reviewed data from our institutional database to identify women aged 30 and older from either the Cancer Prevention Center (CPC) or the Gynecologic Oncology Clinic (GOC) who had a negative result for Pap cervical cytology (SurePath) and a positive result for high-risk HPV (hrHPV) by Hybrid Capture 2 (HC2) or Cervista HPV HR on co-testing during the period 2007-2010. Each patient's follow-up data for up to 8-12 years after the index Pap/HPV co-testing were collected. The cumulative risk of HSIL during the follow up was compared in the two patient populations in our institution (Fisher's Exact test).

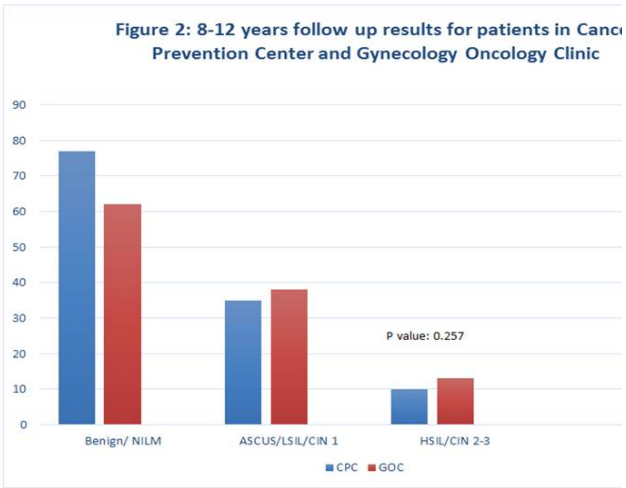
Results: During 2007-2010, hrHPV was detected in 1.5% (196/12,967) of women in CPC and 6.4% (196/3,068) in GOC. A total of 117 women in the CPC (age range 30-79 years, mean 49) and 113 women in the GOC (age range 30-84 years, mean 46) with follow up data were included in the study. The follow-up Pap cytology/biopsy showed that the cumulative risk of HSIL was higher in women in GOC than those in CPC, either for 5-years (8.9% in GOC vs. 5.1% in CPC) or longer than 5-years (11.5% in GOC vs. 6.8% in CPC, although no significant differences were observed between the two populations (Table 1 and 2). During 5-year follow-up, a positive HPV testing result was observed in 32.4% (35/108) women in CPC and 43.8% (39/89) women in GOC.

Figure 1 - 418



CPC: Cancer Prevention Center; GOC: Gynecology Oncology Clinic

Figure 2 - 418



CPC: Cancer Prevention Center; GOC: Gynecology Oncology Clinic

Conclusions: Women with Pap-/HPV+ co-testing results had a distinct risk of HSIL, especially within 5 years follow-up period. Women from high-risk population (GOC) had a higher cumulative risk of HSIL than those from low-risk population. Close follow-up with Pap/HPV co-testing in 5 years is necessary for these women.

419 Utility of Ultrasound-Guided Fine-Needle Aspiration of Neck Lymph Nodes for Initial Staging and Surveillance of Patients with Papillary Thyroid Cancer

Suvra Roy[1], Qiong Gan[1], Savitri Krishnamurthy[1]
[1]The University of Texas MD Anderson Cancer Center, Houston, TX

Disclosures: Suvra Roy: None; Qiong Gan: None; Savitri Krishnamurthy: None

Background: Cytopathologic examination of US-FNA and/or thyroglobulin evaluation in FNA specimens (FNATg) can be used for evaluation of US-FNA from neck lymph nodes (NLN) in patients with papillary thyroid carcinoma (PTC). Data are limited regarding the utility of US-FNA cytology alone for initial staging and surveillance of these patients. The primary objective of our study was to evaluate the performance of US-FNA cytology for guiding clinical management of newly diagnosed PTC and for surveillance of patients with PTC.

Design: We studied patients with PTC who underwent US-FNA for initial staging or surveillance over a one-year period. Sonographers performed US-FNA and FNA smears were evaluated by cytopathologists. US-FNAs cytology were categorized as non-diagnostic, benign/atypical, or suspicious/malignant and correlated with final pathology of lateral neck dissection (LND) and/or clinical follow-up for at least 1 year. The non-diagnostic rate, sensitivity, specificity and accuracy of US-FNA cytology results for initial staging and surveillance of patients with PTC were determined.

Results: We identified 79 cases of PTC, 46 newly diagnosed and 33 under surveillance, who underwent US-FNA of LNDs. The US-FNAs from 46 initial staging cases were categorized as non-diagnostic in 2 (4.3%), benign in 18 (39%), and malignant in 23 (50%) patients. LND was performed in 22/ 23 (95.6%) patients, all of whom had metastasis in one or more LNs. LND was performed in 11 of 18 (61%) patients with a benign result with metastatic disease in 1 of 11 (9%). The 2 patients with non-diagnostic result also underwent LND with metastatic disease in a lymph node in each. The US-FNA cytology in 33 patients as surveillance for PTC was categorized as non-diagnostic in 1 (3%), benign in 23 (69%), atypical in 1 (3%), and malignant in 8 (24%) patients. Only 1/ 8 patients underwent LND which showed metastatic tumor. The non-diagnostic rate, sensitivity, specificity, and accuracy of US-FNA cytology for initial staging were 4.3 %, 95.4%, 95.4%, and 95%; those for surveillance were 3.03%, 100%, 100%, and 100%.

Conclusions: US-FNA cytology of diagnostic specimens can be a highly sensitive and specific test for initial staging and surveillance of patients with PTC. A positive result in the initial staging aids in completion of LND at the time of thyroid surgery. Detection of metastatic disease during surveillance guides selection of patients for repeat surgery.

420 Relation of BK Virus-Positive Urinary Cytology to Urothelial Cell Carcinoma of the Urinary Bladder

Iryna Samarska[1], Faisal Klufah[2], Ghalib Mobaraki[3], Axel zur Hausen[1]
[1]Maastricht University Medical Centre+, Maastricht, Limburg, Netherlands, [2]Maastricht University Medical Center, Maastricht, Limburg, Netherlands, [3]Maastricht UMC+, Maastricht, Netherlands

Disclosures: Iryna Samarska: None; Faisal Klufah: None; Ghalib Mobaraki: None; Axel zur Hausen: None

Background: BK-virus (BKV) has been associated with some rare post-transplant renal cell carcinomas (RCC) and high grade/micropapillary urothelial cell carcinomas (UCC). The aim of this study was to evaluate the relation of BKV-positive urine cytology to UCC.

Design: Thirty patients with BKV-positive urine cytology in the period 2004-2019 were included in this study and analyzed/reviewed by cytology and BKV-immunohistochemistry (IHC) using a monoclonal SV40 antibody known to cross-react with BKV.

Results: Samples were obtained from 25 males with a median age of 68.8 yrs. [range 25-78] and 5 females with a median age of 55 yrs. [range 38-57]. Follow-up period ranged from 12 months to 170 months [median 70.5 months]. All 30 patients showed typical Decoy cells in their urine specimens by cytology. In 27 patients BKV-infection was identified by IHC. In three patients IHC was either inconclusive or no material left for additional analyses, and those patients were not diagnosed with UCC. Five patients (5/30; 16.6%) had history of kidney transplantation, but no history of UCC. Eleven patients did not have history of either kidney transplantation nor UCC (11/30; 36.7%). However, detailed analyses of the medical records revealed that 5 of these received immunosuppressive therapy in the context of other medical conditions. Fourteen patients had a history of (either in situ or invasive) UCC (14/30; 46.7%). In all but one patient the first diagnosis of UCC was made before first presentation with BKV infection (13/14; 92.8%). The period between diagnosis of UCC and BKV-positive cytology ranged between 12 - 156 months (average 53.3months). Nine patients, diagnosed with UCC/CIS, received intravesical treatment (BCG or mitomycin). IHC for BKV was performed on UCC lesions of 13 patients, of which blocks were available in the archive, and IHC was negative in both, primary lesions and metastases.

Conclusions: BKV reactivation is not restricted to immunosuppression but also found in the urine of patients treated for UCC. Of importance, BKV-positivity of urine cytology is more frequently found in patients treated for UCC, maybe partially explained by intravesical treatment possibly reactivating latent BKV. BKV-positivity is also found in patients in the context of immunosuppression. BKV-positivity in UCC-negative patients might be explained by cystitis possibly causing viral reactivation. BKV testing in post-UCC patients is potentially of clinical relevance for the risk assessment of BKV-nephropathy.

421 International Telecytology Consultation: An Academic Institutional Experience

Swati Satturwar[1], Sara Monaco[2], Liron Pantanowitz[3], Juan Xing[4]
[1]Winterville, NC, [2]University of Pittsburgh Medical Center, Pittsburgh, PA, [3]University of Pittsburgh, Wexford, PA, [4]UPMC Shadyside Hospital, Pittsburgh, PA

Disclosures: Swati Satturwar: None; Sara Monaco: None; Liron Pantanowitz: None; Juan Xing: None

Background: Telecytology for second opinion consultation has been limited largely due to technical reasons (e.g. inability to focus on cytology material). Nevertheless, international telecytology consultation was undertaken at our institution in partnership with KingMed (KM) diagnostics in China and The Istituto Mediterraneo per i Trapianti e Terapie ad Alta Specializzazione (ISMETT) in Italy. To overcome issues with scanning cytology slides we adopted a cell block preference for teleconsultation.

Design: Telecytology consultation cases received over a 7.5-year period (January 2012-July 2019) were retrospectively reviewed. Glass slides were scanned using a NanoZoomer in KM (Hamamatsu) or Aperio (Leica) scanner at ISMETT. For KM cases only H&E stained cell blocks were scanned, as well as immunostains requested by consultants. For ISMETT aspirate smears were also scanned in some cases. Patient demographics, case diagnoses, ancillary studies, turn-around-time, and any problems encountered were recorded.

Results: A total of 51 non-gynecologic cases (44 cell block only) were evaluated from 48 patients. The specimens included both non-exfoliative (28) and exfoliative (23) cytology cases: pleural fluids (19), pancreas (14), lymph nodes (6), peritoneal fluids (2) and miscellaneous samples (10). Patients (21 female, 27 male) ranged in age from 18-82 years (mean 64 years). The cytologic diagnoses spectrum included 16 (31.37%) cases positive for malignancy, 7 (13.72%) positive for neoplasm, 6 (11.76%) suspicious for malignancy, 10 (19.6%) atypical, 10 (19.6%) negative for malignancy and 2 (3.92%) non-diagnostic. In 42 (82.35%) cases, immunocytochemistry was requested. Table 1 compares cell block only cases with those that also included smears. Fourteen cases (27.45%) had issues including 9 with deficient clinical information, 4 with slide labeling problems, and 1 that needed a rescan.

Cytology material	Cell block only cases	Aspirates and cell block cases	Aspirates only cases
Referring institution	ISMETT+KM	ISMETT	ISMETT
Number of cases	44 (86.27%)	3 (5.88%)	4 (7.84%)
Definitive positive diagnoses	20 (45.45%)	2 (66.66%)	1 (25%)
Suspicious/atypical diagnoses	12 (27.27%)	1 (33.33%)	3 (75%)
Definitive negative diagnoses	10 (22.72%)	0 (0%)	0 (0%)
Non-diagnostic	2 (4.54%)	0 (0%)	0 (0%)
Turn-around-time	1.5-306 hours	10-168 hours	3-10 hours
Immunostains ordered	39 (88.63%)	3 (100%)	None

Conclusions: Our experience shows that international telecytology for consultation purposes involving non-gynecologic cases is feasible. A meaningful second opinion interpretation was rendered in the majority (64.7%) of cases. Utilizing cell block only for consultation solved focus issues that typically plague cytology whole slide imaging. Cell blocks also provided a valuable source of material for performing immunohistochemistry. Therefore, we strongly recommend the use of cell blocks for second opinion teleconsultation in cytopathology.

422 Ki-67 Proliferation Index in Neuroendocrine Tumors: Can Augmented Reality (AR) Microscopy with Image Analysis Improve Scoring?

Swati Satturwar[1], Joshua Pantanowitz[2], Christopher Manko[3], Sara Monaco[4], Ahmed Ishtiaque[3], Liron Pantanowitz[2]
[1]Winterville, NC, [2]University of Pittsburgh, Wexford, PA, [3]University of Pittsburgh, Pittsburgh, PA, [4]University of Pittsburgh Medical Center, Pittsburgh, PA

Disclosures: Swati Satturwar: None; Joshua Pantanowitz: None; Christopher Manko: None; Sara Monaco: None; Ahmed Ishtiaque: None; Liron Pantanowitz: None

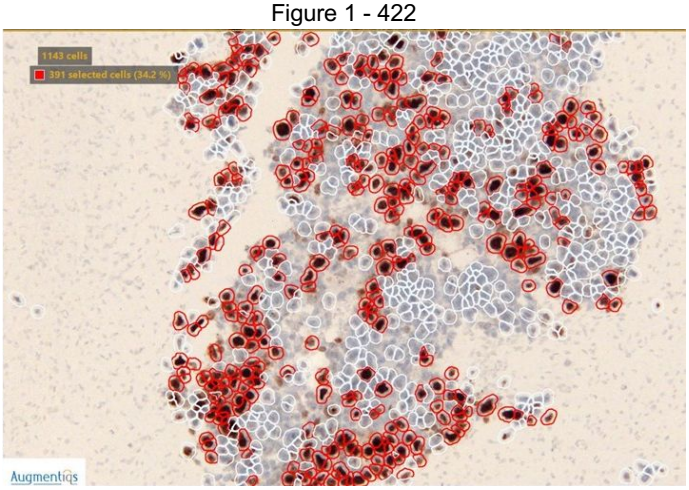
Background: Neuroendocrine tumors (NETs) are frequently diagnosed using fine needle aspiration. Ki-67 index is an essential element in the grading these NETs. However, there are no standardized guidelines for cytopathologists to quantify Ki-67 positivity in cytology material. Different counting methods include 'eye-ball' estimation with a light microscope and manual counting using camera-captured/printed images. Recently, augmented reality (AR) microscopes have become available that enable real-time image analysis with glass slides. Our aim was to compare different scoring methods for calculating the Ki-67 proliferation index in cell block material including the use of an AR microscope.

Design: Ki-67 immunostained slides (Ventana) from 50 NET cases were retrieved (39 pancreas tumors, 11 metastases to liver) of varying grades. The three methods used to calculate Ki-67 index were (1) 'eye-ball' estimation by pathologist using a light microscope, (2) manual counting using camera-captured/printed images and (3) AR microscope (Augmentiqs) with image analysis superimposed on a glass slide (see Figure). Up to three hot spot field of views (FOVs) were counted on each cell block at 20x magnification. For each method Ki-67 score and time spent quantifying were recorded.

Results: The findings comparing different methods are shown in Table 1. While the overall Ki-67 index varied only slightly with the different counting methods, there were marked differences when scoring individual FOVs. A few grade I tumors were upgraded to grade II when using technology to quantify Ki-67. Relative to the simple eye-ball method, the AR microscope method was quicker than employing camera-captured/printed images for scoring.

Scoring method	Eye-ball	Printed image	AR microscope
Ki-67 average score (%)	18% (<1-95)	16.8% (0.1-93.4)	13.5% (0.1-98.7)
WHO Grade I cases	26 (52%)	23 (46%)	25 (50%)
WHO Grade II cases	13 (26%)	16 (32%)	15 (30%)
WHO Grade III & higher cases	11 (22%)	11 (22%)	10 (20%)
Time spent (minutes)	Estimated 1-2	27 (2.3-50)	5.7 (0.5-25)

Figure 1 - 422



Conclusions: The Ki-67 index for NETs in cell block material can vary depending on the method using to score positively stained tumor cells. This difference can affect the grade of these NETs, especially for tumors with lower proliferation indices. Manual counting using camera-captured/printed images was the most laborious and time-consuming method. By superimposing image analysis on glass slides in real-time, the AR microscope method simplifies and speeds up this task for pathologists.

423 Role of Flow Cytometry Studies in the Diagnosis of Classical Hodgkin Lymphoma in Cytology Specimens

Jose Victor Scarpa Carniello[1], Alexander Chan[1], Qi Gao[1], Mikhail Roshal[1], Oscar Lin[1]
[1]Memorial Sloan Kettering Cancer Center, New York, NY

Disclosures: Jose Victor Scarpa Carniello: None; Alexander Chan: None; Qi Gao: None; Mikhail Roshal: None

Background: The diagnosis of classical Hodgkin Lymphoma (cHL) traditionally requires morphological and immunohistochemical confirmation with tissue specimens. More recently, few studies have demonstrated the ability of flow cytometry studies (FCS) to identify Hodgkin cells, which could be helpful in cases with limited material available including cytology specimens. These small specimens have a very limited number of neoplastic cells for a complete immunohistochemical workup, which can prevent a definite diagnosis of cHL, thus requiring a re-biopsy and treatment delay. We report our institutional experience using FCS in cytology specimen for the diagnosis of cHL.

Design: Patients with suspected hematological diseases from a major tertiary cancer center that underwent a biopsy including cytology specimens and dedicated FCS panel for cHL were retrospectively identified. The panel of antibodies used for FCS included CD5, CD15, CD20, CD30, CD40, CD45, CD64, CD71 and CD95. The cytology and FCS reports were analyzed and correlated with the corresponding surgical specimens to analyze the sensitivity and specificity of FCS in the detection of cHL in cytology specimens.

Results: A total of 584 patients meeting the above criteria were identified between the years of 2015 and 2018 with an average age of 55 years. Of these 584 patients, 122 patients had a confirmed histological diagnosis of cHL and appropriate FCS testing. The FCS diagnosis among these 122 patients were positive for cHL (n=69), suspicious (n=10), negative (n=20) and non-diagnostic (n=23) respectively. The cases were considered non-diagnostic due to low cellularity. The sensitivity and specificity of the flow cytometry in the diagnosis of cHL using cytology specimens and FCS were 79% and 99%, respectively.

Conclusions: Our results demonstrated that FCS have very high specificity to diagnose cHL when appropriate antibodies are used and there is adequate cellularity. A cytology specimen morphologically consistent with cHL supported by FCS is sufficient for a definite diagnosis and patient management without the need for histological confirmation. A negative FCS result would require histological and immunohistochemical work up of a tissue sample to confirm a diagnosis of cHL.

424 Lung and Mediastinal EBUS Fine Needle Aspiration Biopsy in Young Adults

Atsuko Seki[1], Deborah Chute[2]
[1]Cleveland Clinic Foundation, Cleveland, OH, [2]Cleveland, OH

Disclosures: Atsuko Seki: None; Deborah Chute: None

Background: Endobronchial ultrasound guided transbronchial needle aspiration (EBUS-TBNA) is used to investigate pulmonary nodules, mediastinal lymphadenopathy, and mediastinal masses in both malignant as well as non-malignant etiologies. EBUS-TBNA is most commonly used in the diagnosis and staging of patients with non-small cell lung cancer in the middle age and elderly population. Since lung cancer is uncommon in young adults, it would be assumed that there are a distinct disease population and clinical background in young adults who undergo EBUS-TBNA. However, this population has not been well studied yet.

Design: We identified all the EBUS-TBNA cases performed in young adults (aged 18-39) from 1/1/2008 to 12/31/2018 in our institution. Cytology diagnosis were correlated with concurrent/subsequent histologic diagnosis and clinical decisions. A final patient classification was created based on the worst cytologic or histologic diagnosis (benign, low-grade, or malignant), with the exception of atypical cytology with subsequent long clinical follow-up with no evidence of malignancy, who were considered benign. All discordant cases and positive/suspicious cases with available slides were re-reviewed together by the authors to confirm the diagnosis.

Results: 257 EBUS-TBNA were performed in 249 young adults (mean age of 31.2). The majority of indications were lymphadenopathy and lung nodule/mass. Final cytologic interpretations included 214 (83%) benign, 14 (5%) atypical, 5 (2%) low grade neoplasm (carcinoid tumor), and 15 (6%) malignant cases. The final patient classification was 214 (86%) benign, 6 (2%) low grade, 29 (12%) malignant. Discordant results were found in 9% of cases, most frequently due to sampling error (41.7%). Of 214 benign patients, 50% had granulomatous disease, with sarcoidosis most common, followed by Histoplasmosis. Of 29 patients with a final malignant classification, the distribution of malignancies is provided in the table. Ultimately, 12 patients (41%) with a malignant diagnosis died during follow-up (average length of follow-up 32 months).

Clinical findings		Total case number = 257
Age, mean (range)		31.2 (18-38)
Male to Female Ratio		0.9
Imaging results	Lymphadenopathy	162 (63.0%)
	Lung nodule/mass	136 (52.9%)
	Ground glass opacities/infiltration/consolidation	32 (12.5%)
	Hilar/paratracheal/mediastinal mass	20 (7.8%)
	Cavitary lesion	11 (4.3%)
	Endobronchial lesion	3 (1.2%)
Cytology		
Location of EBUS/TBNA	Lymph node	214 (83.3%)
	Lung	48 (18.7%)
	Mediastinum	9 (3.5%)
Cytology results	Non-diagnostic	9 (3.5%)
	Negative/benign	214 (83.3%)
	Granuloma	116 (45.1%)
	Atypical/suspicious	14 (5.4%)
	Low grade neoplasm	5 (1.9%)
	Malignant	15 (5.8%)
Discordant cases		24 (9.3%)
	Sampling error	10 (41.7%)
	Volume	8 (33.3%)
	Interpretation	4 (16.7%)
	Slides unable to review	2 (8.3%)

		Final Histology (worst of any biopsies/resections)			
		Negative	Atypical	Low grade	Malignant
Composite Cytology	Non-diagnostic	5	0	0	2
	Negative/benign	86	0	1	3
	Atypical/suspicious	4	0	0	10
	Low grade neoplasm	0	0	5	0
	Malignant	3	0	0	5

Final patient classification	Total patient number = 249
Benign	214 (85.9%)
Granulomatous disease (including Histoplasma)	124 (49.8%)
Cyst	7 (2.7%)
Other infection	12 (4.7%)
Langerhans cell histiocytosis	1 (0.4%)
Hyaline vascular Castleman's disease	1 (0.4%)
Low grade	
Carcinoid tumor	6 (2.4%)
Malignant	29 (11.6%)
Primary lung	11 (4.4%)
Adenocarcinoma	5 (2.0%)
Squamous cell carcinoma	3 (1.2%)
Other	3 (1.2%)
Hematologic	6 (2.4%)
Non-Hodgkin	3 (1.2%)
Hodgkin	3 (1.2%)
Metastasis	12 (4.8%)
Sarcoma	5 (2.0%)
Colorectal	2 (0.8%)
One each of breast, cervix, tongue, larynx, and unknown primary	

Conclusions: In young adults, EBUS-TBNA was most frequently performed to evaluate lymphadenopathy and lung nodules, and granulomatous disease was the most common benign finding. Although rare, primary lung malignancies do occur in young adults, along with metastasis from a variety of other sites, sarcoma being the most common pathology.

425 A Combination of BAP1, 5-HMC and MTAP Immunohistochemical Staining in Malignant Mesothelioma Effusions

Maryam Shahi[1], Tatjana Antic[2], Carrie Fitzpatrick[2], Aliya Husain[2], Thomas Krausz[3]
[1]Johns Hopkins Medical Institutions, Baltimore, MD, [2]The University of Chicago, Chicago, IL, [3]University of Chicago Hospital, Chicago, IL

Disclosures: Maryam Shahi: None; Tatjana Antic: None; Carrie Fitzpatrick: None; Aliya Husain: None; Thomas Krausz: None

Background: Inability to assess invasion as well as wide range of reactive atypia, make the cytologic diagnosis of malignant mesothelioma (MM) particularly challenging. Yet, cytology is an invaluable tool for primary diagnosis or follow up of patients with such diagnosis.

In histologic sections, loss of BAP1 protein expression by immunohistochemistry (IHC) and homozygous deletion of p16/CDKN2a by FISH are highly specific and sensitive for MM. MTAP IHC staining has shown a high concordance rate with homozygous deletion of p16/CDKN2A gene. A combination of MTAP and BAP1 IHC in malignant pleural mesothelioma yielded a sensitivity of 77.8%. Recently, loss of 5-hmC has shown to have a high sensitivity and specificity for diagnosis of malignant mesothelioma on histologic specimens. This study was undertaken to assess the usefulness of these 3 markers in diagnosing MM on effusion cytology.

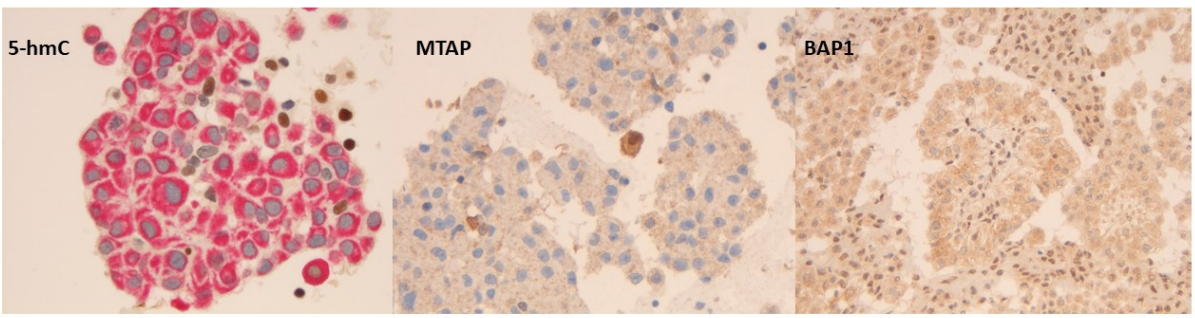
Design: BAP1, MTAP and 5-hmC/CAM5.2 IHC were performed on [HA[1] [MS2] effusion cell blocks (pleural, pericardial and peritoneal) suspicious for or diagnostic of MM, with subsequent confirming biopsy or resection. p16 fluorescent in-situ hybridization (FISH) was performed on either cell block material or histologic specimens.

IHC was scored for loss of BAP1 (nuclear), 5-hmC (nuclear, in >50% of CAM5.2 positive cells) and MTAP (cytoplasmic) immunoexpression and sensitivity, specificity, PPV and NPV of each and all markers in combination were calculated.

Results: 108 cytologic samples from 91 patients (F:M = 1:3) with an age range of 27-89 year (median: 67), were found (2005 -2019). 82 cases had follow up biopsy/resection including 62 epithelioid and 20 biphasic types. MM cell block material and benign reactive mesothelial proliferation histologic samples were stained for BAP1 (27/21), MTAP (27/19) and 5-hmC/CAM5.2 (27/9) IHC. MTAP IHC and CDKN2A FISH had a concordance rate of 90.5% (n=27). Sensitivity and specificity of BAP1, MTAP and 5-hmC were as follows: 77.8%/90.5%, 33.5%/100% and 66.5%/100%, respectively. BAP1+5-hmC and all markers in combination had the highest sensitivity and specificity: 89%/93.5% and 92.5%/93.5%, respectively.

	BAP1	MTAP	5-hmC	BAP1 + MTAP	BAP1 + 5-hmC	MTAP + 5-hmC	BAP1 + MTAP + 5-hmC
Sensitivity	77.78%	33.33%	66.67%	85.19%	88.89%	85.19%	92.59%
Specificity	90.48%	100.00%	100.00%	90.48%	93.33%	93.33%	93.33%
PPV	91.30%	100.00%	100.00%	92.00%	92.31%	100.00%	92.59%
NPV	76.00%	52.63%	50.00%	82.61%	90.32%	87.50%	93.33%

Figure 1 - 425



Conclusions: A combination of BAP1, 5-hmC and MTAP IHC or only BAP1 and 5-hmC IHC in cell blocks from pleural effusions appear to be a reliable method for diagnosis of MM. Cytology is limited in detecting sarcomatoid MM, hence diminishing the benefit of using MTAP IHC (more sensitive for detection of sarcomatoid MM) on cytologic preparations.

426 An 8-Year Retrospective Review of Clinical Outcomes in Patients with "Atypical" Biliary Brushings: Stent Presence does not Affect Subsequent Rates of Malignancy, but does Affect Pathologists' Interpretation

Minqian (Jasmine) Shen[1], Jordan Reynolds[2], Maria Luisa Policarpio-Nicolas[1], Isaac Briskin[3], Kelsey McHugh[4]
[1]Cleveland Clinic Foundation, Cleveland, OH, [2]Cleveland Clinic, Cleveland, OH, [3]Cleveland Clinic Quantitative Health Sciences, Cleveland, OH, [4]Cleveland Clinic, Lakewood, OH

Disclosures: Minqian (Jasmine) Shen: None; Jordan Reynolds: None; Maria Luisa Policarpio-Nicolas: None; Isaac Briskin: None; Kelsey McHugh: None

Background: Biliary brushing of suspected pancreaticobiliary lesions has a notoriously low diagnostic sensitivity. Further confounding, the presence of biliary stents can cause reactive changes that may mimic malignancy. We herein conduct an 8 year retrospective review of a single institution's "atypical" biliary brush cytology diagnoses rendered on specimens collected via endoscopic retrograde cholangiopancreatography (ERCP).

Design: Electronic review of the pathology files (CoPathPlus; Cerner Corp.) was conducted from 01/01/2010 to 12/31/2017 for all biliary brush cytology specimens obtained via ERCP and diagnosed as "atypical." There were 5 interpretive categories: atypical, suspicious (AS); atypical, cannot exclude malignancy (ACE); atypical, favor reactive (AFR); atypical, unspecified (AU); atypical, rare/limited (ARL). The presence or absence of a stent was documented. Associated clinical follow-up including repeat cytology, subsequent surgical pathology, and subsequent radiology were obtained through review of the electronic medical record (Epic; Epic Systems Corp.). Follow-up results were categorized as follows: positive for malignancy, negative for malignancy, persistently atypical and lost to follow-up.

Results: There were 337 "atypical" ERCP biliary brushing specimens and 263 (78.0%) had clinical follow-up. 148 (43.9%) had a stent in place prior to collection; 189 (56.1%) did not. Categorization is as follows: 53 (20.2%) AS, 19 (7.2%) ACE, 81 (30.8%) AU, 47 (17.9 %) ARL, 63 (24.0%) AFR. Follow-up results demonstrate the following rates of malignancy: 48/53 (90.5%) AS, 16/19 (84.2%) ACE, 50/81 (61.7%) AU, 33/47 (70.2%) ARL, 28/63 (44.4%) AFR. "Atypical" biliary brushings have a positive predictive value (PPV) of 66.5%. When the two "atypical" categories that convey highest concern for malignancy (AS, ACE) are isolated, PPV improves to 88.9%. When a stent is present, more cases were interpreted as AFR rather than AS (p=0.02) despite similar rates of malignancy on follow-up (Table 1).

Brushing Interpretation	Malignant	Persistently Atypical	Negative	% Malignant
Stent Present				
AS	13	2	0	86.7
ACE	7	0	2	77.8
ARL	9	0	5	64.3
AFR	17	6	19	40.5
Total	67	10	36	59.3
Stent Absent				
AS	35	3	0	92.1
ACE	9	0	1	90.0
ARL	24	3	6	73.7
AU	28	6	14	58.3
AFR	11	1	9	52.4
Total	107	13	30	71.3

Conclusions: With presence of biliary stent, cytopathologists are more likely to favor reactive atypia over malignant despite no statistically significant difference in malignancy on follow-up. When diagnostic material is insufficient to achieve a definitively positive interpretation, the use of a standardized set of "atypical" categories may provide more meaningful information to clinicians actively constructing intervention/follow-up plans for patients with pancreaticobiliary lesions.

427 MicroRNA Profile Comparison between Noninvasive Follicular Thyroid Neoplasms with Papillary-Like Nuclear Features, Papillary Thyroid Carcinoma and Benign Thyroid Lesions in FNA Smears

Alexander Shtabsky[1], Asia Zubkov[1], Gila Lithwick-Yanai[2], Hila Benjamin[3], Sarit Tabak[2], Danit Lebanony[2], Eti Meiri[2]
[1]Tel Aviv Sourasky Medical Center, Tel Aviv, Israel, [2]Rosetta Genomics, Ltd, Rehovot, Israel, [3]Rosetta Genomics, Inc, Philadelphia, PA

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Background: There are a few works in the literature that compare noninvasive follicular thyroid neoplasms with papillary-like nuclear features (NIFTP) miRNA profile with benign and malignant thyroid lesions in the histological (surgical) material. The aim of our research

was to assess the possibility of diagnosing of NIFTP in stained cytological smears obtained from thyroid fine needle aspiration (FNA), based on microRNA analysis.

Design: The study was performed on routinely prepared FNA smears of 138 thyroid lesions with histologically confirmed diagnosis, using archive material. The histological (surgical) material of the study was re-examined; it included 27 PTC (classic, follicular and mixed variants), 9 NIFTP and 102 benign lesions. The deidentified Diff-Quick, May-Grunwald-Giemsa and Papanicolaou-stained conventional smears were submitted for microRNA analysis by qPCR and microarray; more than 2000 miRNAs were tested. The cases were divided into three groups: papillary thyroid carcinoma (PTC), NIFTP and benign (follicular and Hurthle cell adenomas, colloid and adenomatoid nodules, Graves' disease, lymphocytic and Hashimoto's thyroiditis), and the miRNA expression profiles were compared.

Results: Using microarrays, several markers were found to differ between the groups, especially hsa-miR-146b-5p and hsa-miR-222-3p. These markers showed intermediate expression levels in NIFT-P, relative to PTC and benign, being the highest for PTC and lowest for the benign lesions. The difference of NIFT-P relative to benign was significant ($p=3e-10$, $p=0.002$, respectively), whereas the difference relative to PTC did not meet statistical significance ($p>0.05$). Similar results were obtained using qPCR, where these and additional markers were statistically significantly different between NIFT-P and benign but not between NIFT-P and PTC.

Conclusions: Our results demonstrate the possibility of distinguishing between NIFTP and benign thyroid lesions in stained cytological smears by miRNA profile, specifically hsa-miR-146b-5p and hsa-miR-222-3p. Since NIFTPs are most often diagnosed as indeterminate by thyroid FNA (Bethesda class III, IV or V), miRNAs expression levels can improve a decision making process in these cases in order to avoid unnecessary surgery.

At the same time the differences in expression of the aforementioned miRNAs between NIFTP and PTC were not statistically significant.

Further works on the larger volume of diverse cytological material are necessary to confirm and extend the study results.

428 The Reporting Rates of HSILs and their HPV Testing and Histologic Follow-Up Results: A Comparison between ThinPrep and SurePath Preparations from One Single Academic Institution

William Sinclair[1], I-sanna Gibbons-Fideler[2], Rulong Shen[1], Zaibo Li[1]
[1]The Ohio State University Wexner Medical Center, Columbus, OH, [2]The Ohio State University, Columbus, OH

Disclosures: William Sinclair: None; I-sanna Gibbons-Fideler: None; Rulong Shen: None; Zaibo Li: None

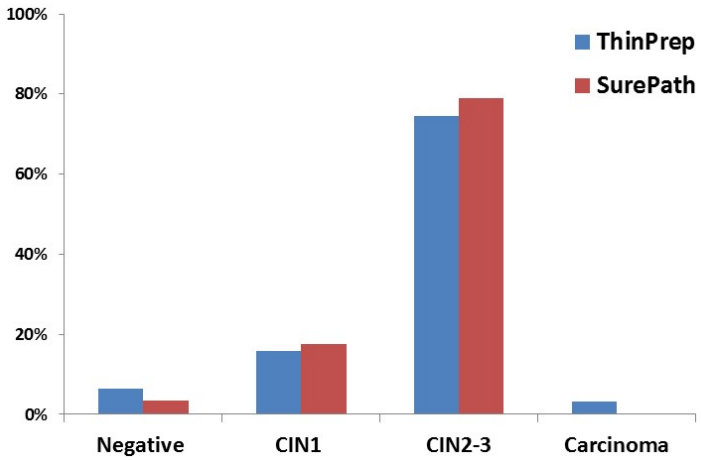
Background: Conventional Papanicolaou (Pap) test has mostly been replaced by liquid-based cytology (LBC) tests [mainly SurePath (SP) and ThinPrep (TP)] for cervical cytology because of their higher sensitivity. However, the comparison of sensitivity and efficacy between these two methods is lacking. The aim of our study was to compare the HSIL reporting rates, HPV positive rates and histological outcome between these two methods.

Design: In our institution, both TP and SP were utilized during the period between January 2014 and June 2017. A retrospective search was conducted to identify patients with HSILs from 40,487 LBCs (15,382 TP and 25,105 SP). Roche Cobas HPV testing and histologic follow-up results were collected.

Results: The reporting rates of HSILs for TP (0.81%) or SP (0.37%) were within the CAP benchmark ranges, but the reporting for TP was significantly greater than that for SP ($p<0.0001$). The HPV-positive rates were 96% and 90% in TP-HSILs and SP-HSILs, respectively, with no statistical significance. Sixty-three (50%) TP-HSILs and 57 (61%) SP-HSILs had histologic follow-up (Table 1). High-grade squamous intraepithelial lesions/carcinomas were identified in 78% (49/63) of TP-HSILs and 79% (45/57) of SP-HSILs, with no statistical significance (Figure 1).

ThinPrep								
	HSIL HPV+		HSIL HPV-		HSIL no HPV		Total HSIL	
	#	%	#	%	#	%	#	%
Negative	2	7%	0	0%	2	6%	4	6%
CIN1	5	18%	0	0%	5	15%	10	16%
CIN2-3	19	68%	1	50%	27	79%	47	75%
Carcinoma	2	7%	0	0%	0	0%	2	3%
AIS	0	0%	0	0%	0	0%	0	0%
ADC	0	0%	0	0%	0	0%	0	0%
Other	0	0%	0	0%	0	0%	0	0%
With FU	28	65%	1	50%	34	43%	63	50%
W/O FU	15	35%	1	50%	46	58%	62	50%
Total	43		2		80		125	
SurePath								
	HSIL HPV+		HSIL HPV-		HSIL no HPV		Total HSIL	
Negative	1	6%	0	0%	1	3%	2	4%
CIN1	3	17%	1	33%	6	17%	10	18%
CIN2-3	14	78%	2	67%	29	81%	45	79%
Carcinoma	0	0%	0	0%	0	0%	0	0%
AIS	0	0%	0	0%	0	0%	0	0%
ADC	0	0%	0	0%	0	0%	0	0%
Other	0	0%	0	0%	0	0%	0	0%
With FU	18	69%	3	100%	36	56%	57	61%
W/O FU	8	31%	0	0%	28	44%	36	39%
Total	26		3		64		93	

Figure 1 - 428



Conclusions: TP preparation detected significant more HSILs than SP preparation. There was no significant difference in HPV-positive rates or histologic follow-up outcomes between TP-detected HSILs and SP-detected HSILs.

429 Inter-Observer Variability in Programmed Death-Ligand 1 (PD-L1) Immunohistochemistry Scoring in Non-Small Cell Lung Cancer (NSCLC) Cytologic Specimens

William Sinclair[1], Peter Kobalka[2], Rongqin Ren[3], Boulos Beshai[4], Abberly Lott Limbach[1], Lai Wei[5], Zaibo Li[1]
[1]The Ohio State University Wexner Medical Center, Columbus, OH, [2]The Ohio State University, Upper Arlington, OH, [3]The Ohio State University Wexner Medical Center, Westerville, OH, [4]Roswell Park Comprehensive Cancer Center, Buffalo, NY, [5]The Ohio State University, Columbus, OH

Disclosures: William Sinclair: None; Peter Kobalka: None; Rongqin Ren: None; Boulos Beshai: None; Abberly Lott Limbach: None; Lai Wei: None; Zaibo Li: None

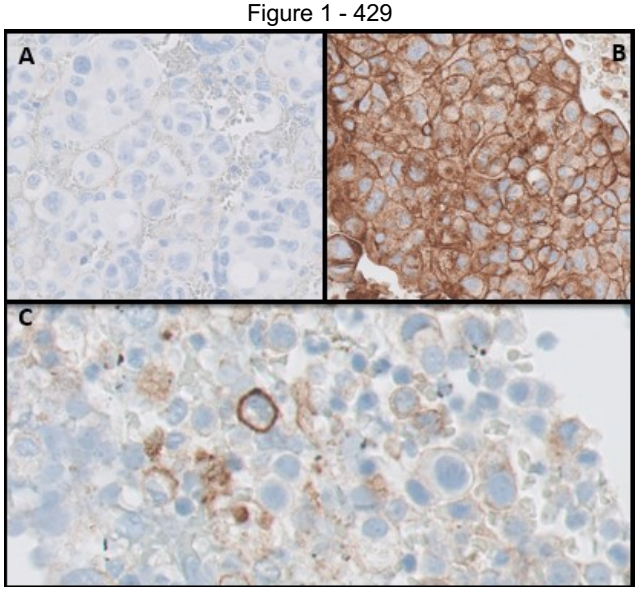
Background: The evaluation of PD-L1 expression in NSCLC is becoming increasingly important given the effectiveness of PD-L1 inhibitors such as pembrolizumab. Although cytologic specimens have been shown to be compatible with surgical specimens in the evaluation of PD-L1 immunohistochemistry (IHC), evidence of the reproducibility of PD-L1 in cytologic specimens is lacking. The aim of this study is to evaluate inter-observer variability in PD-L1 IHC in cytologic specimens.

Design: PD-L1 IHC was performed on 86 NSCLC cytology specimens using Dako PD-L1 IHC 22C3 pharmDx (clone 22C3) according to manufacturer's protocol. The glass slides were digitally scanned using Philips UltraFast Scanner at 40x magnification and the whole slide images (WSI) were read by 5 separate pathologists, including one resident pathologist. Each case was given a Tumor Proportion Score (TPS) and the results were compared between the observers using Spearman correlation coefficient and Cohen's Kappa coefficient. The inter-observer concordance was assessed using 1% and 50% as cutoffs. Figure 1 provides examples of cell blocks stained with PD-LI IHC with <1% staining (A), >50% staining (B), and 1-50% staining (C).

Results: The TPSs were highly correlated among the observers in cytologic specimens (Spearman correlation coefficient, 0.86 – 0.94, p-value <0.0001). Using >1% as a cutoff, inter-observer variability measured by Cohen's Kappa coefficient ranged from 0.49 to 0.83, consistent with moderate to substantial agreement. With a cutoff of >50%, the kappa values ranged from 0.63 to 0.90 indicating substantial to almost perfect agreement (Table 1).

1% Kappa coefficient (p-value)	5	1	2	3
1	0.5982 (.0002)			
2	0.4948 (<.0001)	0.7662 (1)		
3	0.5903 (.0045)	0.8353 (.1025)	0.7071 (.2059)	
4	0.7074 (.0114)	0.7549 (.0196)	0.6671 (.0209)	0.8137 (.2568)
50% Kappa coefficient (p-value)	5	1	2	3
1	0.7595 (.0047)			
2	0.6307 (.0005)	0.8525 (.0455)		
3	0.7761 (.0339)	0.8685 (.3173)	0.7268 (.0339)	
4	0.8046 (.0588)	0.9034 (.0833)	0.7698 (.0082)	0.8502 (.6547)

Figure 1 - 429



Conclusions: Inter-observer agreement for PD-LI IHC staining on cytology specimens was highly correlated among the observers. However, a significantly better agreement among observers was seen using 50% as the cutoff rather than using 1%. These findings suggest that TPSs using PD-L1 IHC on cytology specimens are reproducible; however, special attention is required when the TPS is near the 1% cutoff.

430 Combining RAS Mutational Status with Cytologic Features Improves Risk Stratification of Indeterminate Thyroid Nodules

Sam Sirotnikov[1], Joshua Kagan[2], Daniel Lubin[3], Michelle Reid[4], Melinda Lewis[1], Amy Chen[3], Qiuying (Judy) Shi[3]
[1]Emory School of Medicine, Atlanta, GA, [2]Emory University, Decatur, GA, [3]Emory University, Atlanta, GA, [4]Emory University Hospital, Atlanta, GA

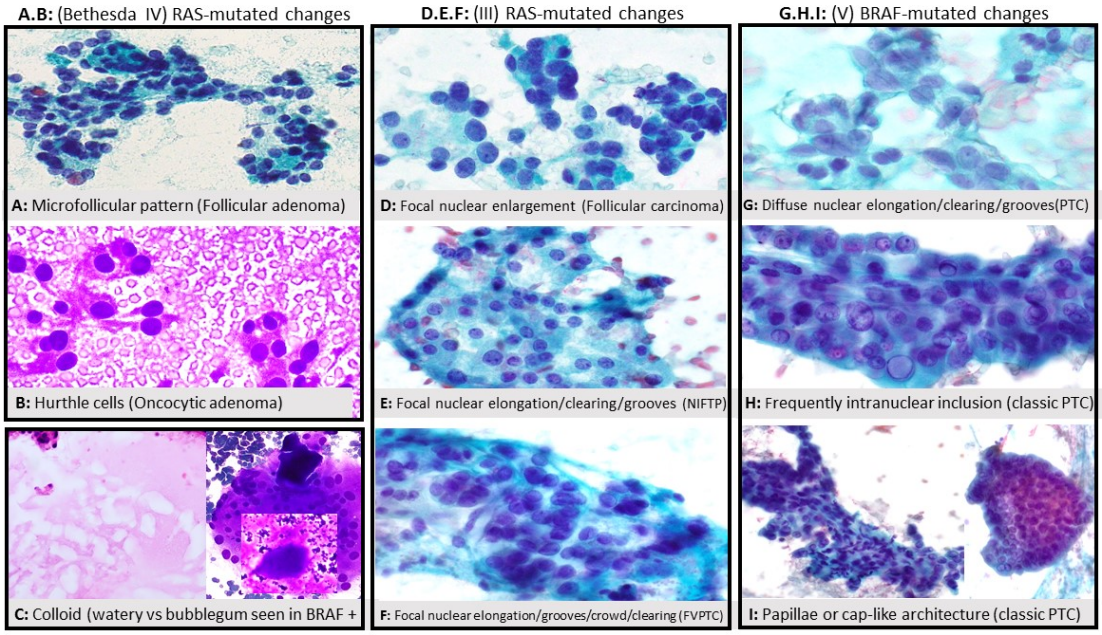
Disclosures: Sam Sirotnikov: None; Joshua Kagan: None; Michelle Reid: None; Melinda Lewis: None; Amy Chen: None; Qiuying (Judy) Shi: None

Background: Molecular tests are increasingly used in the workup of thyroid lesions with indeterminate cytology. ThyroSeq v3 (Tv3) Genomic Classifier is a next-generation sequencing test that can risk stratify such lesions. We examined if different *RAS* mutations might be associated with a unique cytomorphology.

Design: We identified 50 indeterminate thyroid FNAs from 2017-2019 with *RAS* mutations and 6 with *BRAF* mutations. 37 were classified as atypia of undetermined significance (Bethesda III), 9 as suspicious for follicular neoplasm (IV), and 10 as suspicious for malignancy (V). Mutations were given low, intermediate, intermediate-high and high risk of malignancy (ROM) from the Tv3 report. Semi-quantitated cytomorphologic features (cellularity, colloid, nuclear crowding, size, elongation, grooves, intranuclear inclusion, chromatin clearing, microfollicular pattern, irregular nuclear contour, oncocytic cells) [see image attached] were correlated with molecular results and subsequent resection.

Results: The FNAs from patients with *RAS* mutations were given 14% low, 42% intermediate, 42% intermediate-high, and 2% high ROM; All 6 *BRAF* mutated FNAs were given high ROM. Resection data was available in 36/56 (64%) of cases. Of 32 *RAS*-mutated lesions, 20 (62%) were malignant and had a dominant follicular architecture, while 12 (38%) were benign, including 4 NIFTPs. All 4 *BRAF*-mutated lesions with follow-up showed classic PTC. Nodules with *NRAS/TERT* and *NRAS/EIF1AX* mutations showed classic PTC with predominant follicular growth. The *RAS* subtypes demonstrated somewhat overlapping cytomorphologic characteristics. Compared to *RAS* mutated lesions, those with *BRAF* mutations showed more suspicious changes and markedly increased intranuclear inclusions and cap-like/papillary architecture (both seen in 83% of *BRAF*-mutated lesions vs just 10% and 2% of *RAS*-mutated lesions, respectively).

Figure 1 - 430



* According to each cytomorphological feature above, scores are assigned as 0, 1, 2, or 3 based on severity

Conclusions: The majority (62%) of *RAS* mutated resected lesions were malignant. Unlike *BRAF* mutated lesions with more prominent suspicious changes, overlapping cytologic features were seen in *RAS* mutated subtypes. Combining cytomorphology with ancillary molecular tests will help to triage the indeterminate thyroid nodules.

431 A Correlative Analysis of The Paris System (TPS) for Reporting Urine Cytology: Results from a Large Academic Institution

Justin Snow[1], Qian Wang[2], Wei Sun[3], Yan Shi[3], Aylin Simsir[4], Tamar Brandler[5]
[1]New York University Langone Health, New York, NY, [2]NYU Langone Health, New York City, NY, [3]New York University Langone Medical Center, New York, NY, [4]New York University School of Medicine, Edison, NJ, [5]NYU Langone Health, Highland Park, NJ

Disclosures: Justin Snow: None; Qian Wang: None; Wei Sun: None; Yan Shi: None; Aylin Simsir: None; Tamar Brandler: None

Background: TPS is a reporting system that includes specific diagnostic categories and cytologic criteria for the accurate diagnosis of high-grade urothelial carcinoma (HGUC). Its success is dependent on its acceptance and widespread use by the cytology and urology communities. Since its development in 2013, institutions have been transitioning to TPS in an effort to standardize terminology and increase the sensitivity of diagnosing HGUC. We present our data comparing TPS diagnoses (PD) to the traditional reporting system (TD) in correlation with the gold standard surgical pathology diagnosis (SD).

Design: A search of the pathology database was conducted on urine cytology specimens from adult patients from 7/1/2014-6/30/2016 (TD) and 7/1/2017-6/30/2019 (PD). 454 cytology specimens from 382 patients were found to have corresponding urinary tract SD within 90 days and were included in the study. 192/454 urines were from prior to TPS implementation; 262/454 were from after TPS implementation. TD included: Positive for malignancy/urothelial carcinoma (POS), suspicious for malignancy/urothelial carcinoma (SUS), atypical, low-grade urothelial neoplasia/carcinoma (LG), and negative for malignancy (NEG). TD and PD were compared to their corresponding SD.

Results: 34/41 (83%) of HGUC were correctly identified using PD compared to 36/49 (73%) using TD. 15/23 (65%) of SHGUC correlated with HGUC on SD using PD compared with 13/16 (81%) with TD. Rates of “Atypical” diagnoses were decreased from 82/192 (42%) with TD to 95/262 (36%) with PD while the risk of malignancy (ROM) with “Atypical” diagnosis increased using PD from 33% to 36%. LG was identified on cytology in 1/43 (2%) using TD and 3/65 (5%) with PD. In both TD and PD, LG cytologic diagnosis had 100% specificity. 31/65 (48%) of LGUN were correctly categorized as NHGUC using PD while 10/43 (23%) were NEG in TD. (Table 1 and Figure 1)

Table 1: Cytohistologic Correlation Results Before (TD) and After (PD) Implementation of TPS

	SD						
TD	**HGUC / HGPAP / UCIS**	**SHGUC / SCIS**	**ATYPICAL / DYSPLASTIC**	**LGUN / PAPILLOMA / PUNLMP**	**BENIGN / NEGATIVE**	**OTHER MALIGNANCIES**	**TOTAL**
POS	36	0	3	5	4	1	**49**
SUS	13	0	0	2	1	0	**16**
ATYPICAL	27	0	7	25	19	4	**82**
LG	0	0	0	1	0	0	**1**
NEG	9	0	1	10	24	0	**44**
	85	**0**	**11**	**43**	**48**	**5**	**192**
	SD						
PD	**HGUC / HGPAP / UCIS**	**SHGUC / SCIS**	**ATYPICAL / DYSPLASTIC**	**LGUN / PAPILLOMA / PUNLMP**	**BENIGN / NEGATIVE**	**OTHER MALIGNANCIES**	**TOTAL**
HGUC	34	0	0	1	5	1	**41**
SHGUC	15	0	1	1	6	0	**23**
ATYPICAL	34	0	5	29	25	2	**95**
LGUN	0	0	0	3	0	0	**3**
NHGUC	18	0	3	31	46	0	**98**
OTHER	0	0	0	0	0	2	**2**
	101	**0**	**9**	**65**	**82**	**5**	**262**

Key: TD = Traditional Reporting Terminology (prior to TPS); PD = TPS Diagnosis; SD = Surgical Pathology Diagnosis; HGUC = High-Grade Urothelial Carcinoma; SHGUC = Suspicious for HGUC; AUC = Atypical Urothelial Cells; LGUN = Low-Grade Urothelial Neoplasia; NHGUC = Negative for HGUC; HGPAP = High-Grade Papillary Urothelial Carcinoma; UCIS = Urothelial Carcinoma in-situ; SCIS = Suspicious for UCIS; PUNLMP = Papillary Urothelial Neoplasm of Low Malignant Potential

Figure 1 - 431

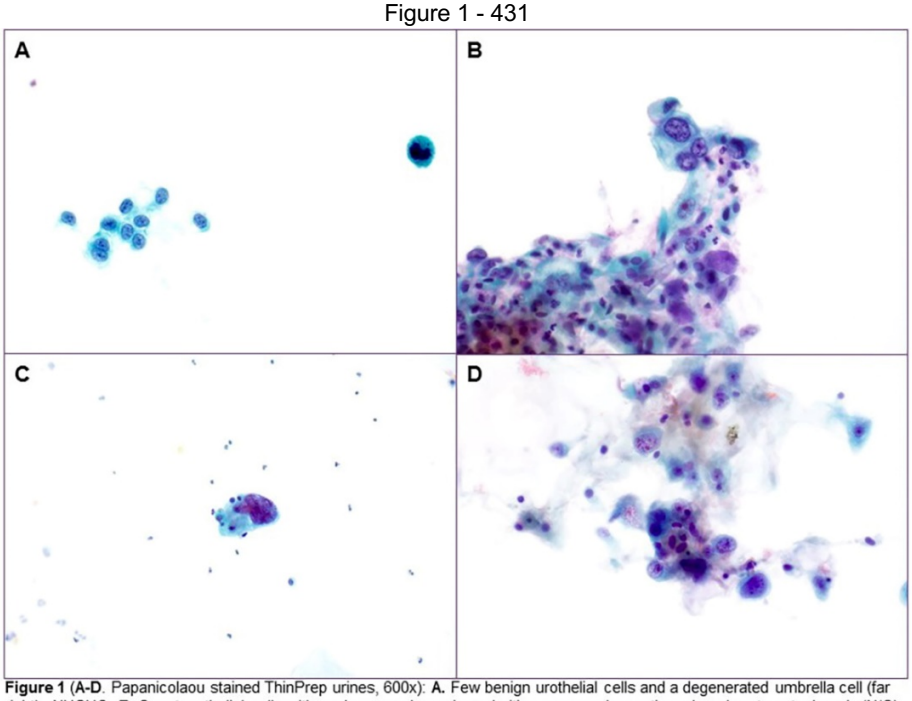


Figure 1 (**A-D**. Papanicolaou stained ThinPrep urines, 600x): **A.** Few benign urothelial cells and a degenerated umbrella cell (far right), NHGUC. **B.** Scant urothelial cells with nuclear membrane irregularities, coarse chromatin and nuclear-to-cytoplasmic (N/C) ratio of > 0.5 but < 0.7, meeting the criteria for AUC using PD. **C.** Large pleomorphic cell with nuclear hyperchromasia, nuclear irregularity and increased N/C ratio > 0.7 consistent with SHGUC. **D.** Malignant urothelial cells seen singly and in groups showing nuclear enlargement, hyperchromasia, coarse chromatin and nuclear irregularities with increased N/C ratio > 0.7, Positive for HGUC.

Conclusions: Implementation of TPS in our laboratory led to a higher accuracy in the cytologic diagnosis of HGUC. Additionally, the "Atypical" rate decreased from 42% to 36% while the ROM showed a modest increase. While 12% of HGUCs diagnosed with TPS were found to be benign on SD, 60% of these cases actually had prior and/or subsequent HGUC/CIS on SD indicating that original PD was in fact concordant. LGUN is difficult to diagnose on cytology, and TPS afforded an increase in NHGUC diagnoses in line with the main goal of the PD- diagnosis of HGUC.

432 Endobronchial Ultrasound-Guided Transbronchial Fine-Needle Aspiration is not an Accurate Predictor of Lymph Node Status in Malignant Pleural Mesothelioma

Linda Song[1], Rachel Fanaroff[2], Allen Burke[2], Paul Staats[3]
[1]University of Maryland Medical Center, Baltimore, MD, [2]University of Maryland, Baltimore, MD, [3]University of Maryland School of Medicine, Baltimore, MD

Disclosures: Linda Song: None; Rachel Fanaroff: None; Allen Burke: None; Paul Staats: None

Background: Current treatment of diffuse malignant pleural mesothelioma (MPM) consists of chemoradiation and/or surgical resection with treatment modality often depending on the stage of the disease. Staging of MPM involves both advanced imaging and surgical modalities. Currently, there is little evaluation of endobronchial ultrasound-guided transbronchial fine-needle aspiration (EBUS-TBNA) in lymph node staging of MPM. This study assesses the correlation of EBUS-TBNA with final MPM surgical resection specimens.

Design: A 5-year retrospective review of cytology and pathology records of patients who have undergone EBUS-TBNA lymph node staging and extended pleurectomy decortication (EPD) of MPM at a single academic institution was performed.

Results: Thirty-two patients underwent both procedures: 25 (78%) men and 7 (22%) women. Mesothelioma subtype was epithelioid in 24 (75%) and biphasic in 8 (25%). Seven patients (22%) had positive EBUS-TBNA findings (ranging from 1-3 nodes per patient) with total positive lymph nodes as follows: station 4 (n=4), station 7 (n=5), station 11 (n=2). Eighteen patients (56%) had positive lymph nodes at EPD (range 1-8 nodes per patient) with total positive nodes as follows: posterior intercostal (n=18), station 9 (n=12), station 7 (n=10), phrenic (n=9), intermammary (n=8), hilar (n=7), other (n=21). There were 10 patients in whom the same nodal stations were negative by EBUS-TBNA but positive by EPD: station 7 (7/10), station 4 (1/10), and both 4 and 7 (2/10). Three patients were positive (n=2; 1 stations 11 and 7, 1 station 4) or suspicious (n=1, station 4) by EBUS-TBNA but negative at EPD. Treating EPD as the gold standard, the overall sensitivity of EBUS-TBNA was 31% and specificity was 92%; station-specific sensitivity was 31% and specificity was 90%.

Conclusions: Lymph node metastases occur in over half of patients with diffuse MPM. EBUS-TBNA does not appear to be a sensitive method for detecting lymph node metastases in MPM. While EBUS can access most mediastinal stations, it cannot sample some nodes frequently involved by MPM, such as posterior intercostal, station 9, phrenic, and inframammary. However, even among sampled lymph nodes sensitivity is poor. Specificity is relatively high. The presence of occasional false positives may be attributable to contamination by inadvertent sampling of adjacent non-nodal tumor.

433 p16 Immunohistochemistry (IHC) in Cell Blocks and Tissue Specimens in Oropharyngeal Squamous Cell Carcinomas (SCC): A Correlation Study

Sharon Song[1], Jalal Jalaly[1]
[1]Hospital of the University of Pennsylvania, Philadelphia, PA

Disclosures: Sharon Song: None; Jalal Jalaly: None

Background: Head and neck squamous cell carcinomas (HNSCC) frequently present as a neck mass without a known primary site, requiring FNA for diagnosis. Human Papillomavirus (HPV) status in oropharyngeal SCC drives prognosis. In 2017, the College of American Pathologists recommended the use of p16 as a surrogate marker for HPV status, with a cutoff of moderate to strong nuclear and cytoplasmic staining in >70% of tumor cells in surgical specimens. However, the preferred method of HPV testing in cytology specimens and p16 positivity cutoff are not established. Here, we report the performance of p16 staining in cell blocks compared to staining in tissue specimens.

Design: All cases of HNSCC presenting as a neck mass and evaluated by FNA from 1/1/14 to 6/30/19 were identified. Cytology cases with a p16 result and a corresponding tissue specimen with p16 IHC were included in the study. Two pathologists blinded to the tissue p16 status assessed the following from cell block material: tumor cellularity (number of clusters with >10 cells), percentage of any nuclear and cytoplasmic staining (quantified in 5% increments if ≤20% of tumor cells staining or 10% increments with >20%), and presence of staining in clusters vs. single cells. Results were compared to the tissue p16 status.

Results: Of 42 HNSCC neck metastases, primary sites included 36 (85.7%) oropharyngeal, 4 (9.5%) non-oropharyngeal, and 2 (4.8%) unknown. Analysis was limited to oropharyngeal and unknown primaries (n=38), and any amount of p16 staining (≥1% of tumor cells) in cell blocks had the best correlation with p16 status in tissue specimens (see Table 1). There were 5 false negatives—2 had no cellularity on the cell block, 2 had low cellularity (1-2 clusters), and 1 had 6 clusters of tumor cells. There was 1 “false positive” (61-70% staining, >10 clusters); however, concurrent HPV DNA PCR testing performed on cytology and surgical material was positive in both specimen types. Staining was present in single cells in 7 (23%) cases, clusters in 14 (45%) cases, both in 8 (26%) cases, and unknown in 2 cases (unavailable for review).

Table 1: p16 IHC in Cell Blocks (CB) and Surgical Pathology (SP)

	All	Oropharyngeal
CB+/SP+	29	28
CB-/SP+	5	5
CB+/SP-	1*	1*
CB-/SP-	3	2
Sensitivity	85.3%	84.8%
Specificity	75%	66.7%
Positive Predictive Value	96.7%	96.6%
Negative Predictive Value	37.5%	28.6%

* HPV DNA PCR was positive in surgical and cell block specimens

Conclusions: Compared to tissue, the cutoff for p16 IHC interpretation in cell blocks is substantially lower and staining may be present in single cells or clusters. In 96.7% of cases, any p16 staining in cell blocks correlated with positive p16 staining in surgical specimens. However, a negative p16 result should prompt repeat p16 testing in tissue specimens, as false negative p16 staining in cell blocks is high.

434 Cytology and Aptima HPV mRNA Screening Test Results Associated with Histological Diagnosis of CIN2/3

Pooja Srivastava[1], Chengquan Zhao[2]
[1]University of Pittsburgh School of Medicine, Pittsburgh, PA, [2]Pittsburgh, PA

Disclosures: Pooja Srivastava: None; Chengquan Zhao: None

Background: To assess cervical screening test performance along with high risk HPV testing in a span of three years preceding the histopathologic diagnosis of cervical intraepithelial neoplasia (CIN) 2/3.

Design: This is a retrospective study in which screening test results of Liquid based cytology (LBC) and Aptima mRNA high-risk HPV testing (hrHPV) were analyzed for 519 patients within a time period of three years preceding the histopathologic CIN2/3 diagnosis.

Results: Total 519 patients data was analyzed for CIN2/3 diagnosis out of which 337 patients had LBC findings within first year: high grade squamous intraepithelial lesion (n=134; 39.7%), low grade squamous intraepithelial lesion (n=34; 10%), atypical squamous cells of undetermined significance (ASC-US) (n=56; 16.6%), atypical squamous cells, cannot exclude high grade squamous intraepithelial lesion (ASC-H) (n=91; 27%), and atypical glandular cells/adenocarcinoma in situ (n=16; 4.7%). 210 patients also had LBC performed at more than 1 year to 3 years before the diagnosis of CIN2/3: at least one abnormal LBC results (n=176; 83.8%), at least one negative LBC result (n=52; 24.7%), both abnormal and negative LBC results(n=18; 8.5%). Out of 337 patients within 1 year of CIN2/3 diagnosis 280 patients had hrHPV cotest results and 274 (97.8%) had positive hrHPV result. 168 patients in period preceding one to three years the CIN2/3 diagnosis had hrHPV cotest results: 147 (87.5%) patients had at least one positive hrHPV test result, 26 (15.4%) patients had at least one negative hrHPV test result and 6 (3.57%) patients had both negative and positive hrHPV test result.

Conclusions: Both recent Pap test and HPV test have high sensitivity for CIN2/3. However, when the same patient population was analyzed for earlier LBC and hrHPV results (1-3 years) preceding their CIN2/3 diagnosis significant number of patients had negative Pap test or negative HPV test results. This data thus highlights the importance of cotesting LBC and hrHPV to enhance detection of precursor lesions and aiding in early diagnosis of CIN2/3.

435 Determining the Significance of Psammoma Bodies in Pelvic Washings: A 10-Year Retrospective Review

Tong Sun[1], Vanda Torous[2]
[1]Mass General Hospital, Boston, MA, [2]Massachusetts General Hospital, Boston, MA

Disclosures: Tong Sun: None; Vanda Torous: None

Background: Pelvic washings (PWs) are relatively common specimens submitted for cytologic examination to exclude metastatic disease in patients undergoing surgery for known malignancies or to exclude microscopic /incidental disease in those undergoing surgery for benign processes or risk reducing surgeries. The finding of psammoma bodies (PBs) in these specimens is a relatively rare occurrence despite the commonality of this specimen type and to date large-scale studies on their cytologic-histologic correlates and thus clinical significance have been limited.

Design: To evaluate the significance of finding PBs in PW specimens, we performed a retrospective search of our institutional pathology archives for all PW specimens noted to contain PBs from 7/1/2009 to 6/30/2019. The findings on the concurrent surgical specimens, including any follow-up surgical specimens, were reviewed.

Results: Over the 10-year period, 123 PW cases showed PBs (Table 1). Over half of the cases (N = 65; 53%) were associated with benign processes, including mesothelial hyperplasia (N = 32; 26%), endometriosis (N = 15; 12%), ovarian cystadenoma/cystadenofibroma (N = 10; 8%), and endosalpingiosis (N = 8; 7%). Twenty-nine cases (24%) demonstrated serous borderline tumors on the surgical specimen. Malignancy was noted in only 29 cases (24%), which included low-grade serous adenocarcinoma (N = 11; 9%), high-grade serous adenocarcinoma (N = 9; 7%), endometrial carcinoma (N = 5; 4%), mixed malignant müllerian tumor (N = 2; 2%), clear cell carcinoma (N = 1; 1%), and mixed serous and endometrial carcinoma (N = 1; 1%). Notably, patients found to have benign processes or borderline disease were significantly younger than patients with malignancies (median ages 50 and 53 vs 65 years, $P_{trend} < 0.0001$). The cytologic interpretation categories were also documented and correlated with the concurrent histopathologic findings, showing that cytologic analysis is highly sensitive and specific in determining the nature of underlying processes (Table 2 and Figure 1).

Table 1. Histopathologic Findings of Pelvic Washings with Psammoma Bodies

Benign (N = 65, median age = 50 years)	
Ovarian cystadenoma/cystadenofibroma	10 (8%)
Mesothelial hyperplasia	32 (26%)
Endosalpingiosis	8 (7%)
Endometriosis	15 (12%)
Borderline (N = 29, median age = 53 years)	
Serous borderline tumor	29 (24%)
Malignant (N = 29, median age = 65 years)	
Low grade serous adenocarcinoma	11 (9%)
High grade serous adenocarcinoma	9 (7%)
Endometrial carcinoma	5 (4%)
Mixed malignant müllerian tumor	2 (2%)
Clear cell carcinoma	1 (1%)
Mixed serous and endometrial carcinoma	1 (1%)
Total	123 (100%)

Figure 1 - 435

Table 2. Cytologic Interpretation Categories and Histopathologic Correlation in Pelvic Washings with Psammoma Bodies

Cytologic \ Histologic N (%)	Benign	Borderline	Malignant	Total
Negative	40 (98%)	1(2%)	0 (0%)	41 (34%)
Atypical	25 (69%)	9 (25%)	2 (6%)	36 (29%)
Neoplastic	0 (0%)	15 (71%)	6 (29%)	21 (17%)
Positive	0 (0%)	4 (16%)	21 (84%)	25 (20%)
Total	65 (53%)	29 (24%)	29 (24%)	123 (100%)

Figure 2 - 435

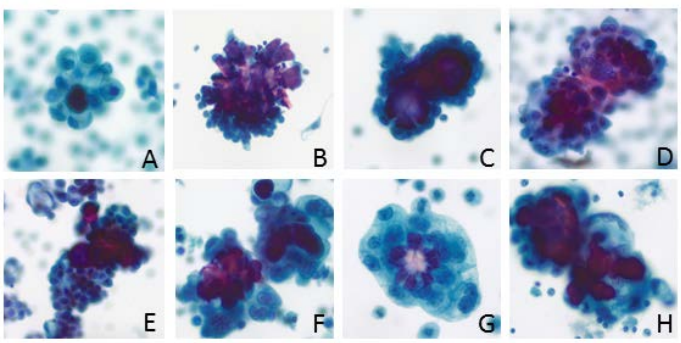


Figure 1. Representative Examples of Pelvic Washings with Psammoma Bodies.
A. Mesothelial hyperplasia **B.** Endosalpingosis **C.** Endometriosis **D.** Serous cystadenoma **E.** Low grade serous carcinoma **F.** High grade serous carcinoma **G.** Clear cell carcinoma **H.** Malignant mixed Müllerian tumor

Conclusions: The majority of PBs noted in PWs were associated with benign processes or borderline tumors, with only about a quarter of the cases associated with malignancies. PBs in PWs from younger patients are more significantly associated with benign processes or borderline tumors.

436 Deep Learning Algorithms for the Identification of Papillary Thyroid Carcinoma on Cytology ThinPrep Images

Simon Sung[1], Jihui Lee[2], Jun Lu[3], Jennifer Kuo[4], Anjali Saqi[4]
[1]New York-Presbyterian/Columbia University Medical Center, New York, NY, [2]Weill Cornell Medical College, New York, NY, [3]Columbia University Mailman School of Public Health, New York, NY, [4]Columbia University Medical Center, New York, NY

Disclosures: Simon Sung: None; Jihui Lee: None; Jun Lu: None; Jennifer Kuo: None; Anjali Saqi: None

Background: Thyroid fine needle aspiration (FNA) has high accuracy for diagnosing benign thyroid nodules vs papillary thyroid carcinoma (PTC) due to distinct cytomorphologic features of PTC. However, some inter- and intra-observer variability exists, and much higher for the indeterminate lesions. Advances in deep learning algorithms in medicine have enhanced the diagnostic accuracy while minimizing subjectivity. We propose applying convolutional neural networks (CNNs) to evaluate the thyroid FNA biopsy samples for predicting the risk of malignancy.

Design: We collected images (both whole slide scans and camera attached to a microscope) from ThinPrep slides. The lesional areas were photographed at 40x magnification and saved as JPEG images. The images were divided into a training set of 302 samples (112 benign, 190 PTC) and a test set of 80 samples (40 benign, 40 PTC) for training CNN and evaluating the performance of fitted CNN, respectively. We exploited the transfer learning properties of CNNs for thyroid cancer diagnosis in cytopathology images. We utilized 6 pretrained CNNs (including VGG-16, VGG-19, Densenet121, AlexNet, Wide ResNet, and MnasNet) as fixed feature extractors and trained added classifiers on the training set. Fitted CNNs were further evaluated and compared on the test set.

Results: We collected a total of 378 FNA biopsy sample images, including those from whole slide scans; 150 Bethesda Category II (benign multinodular goiters) and 228 Bethesda Category VI (Malignant, PTC). A maximum of 87.50% accuracy was achieved by VGG-19 model, which had a specificity of 80.00% and sensitivity of 95.00% in the prediction of PTC. The positive and negative predictive values were 82.61% and 94.12% respectively.

Conclusions: In our proof of concept study, we demonstrate the potential utility of deep learning algorithms for differentiating between benign and malignant lesions. Our study is limited by a small sample size. A larger number of samples will likely improve accuracy. Moreover, by introducing a wider variety of nodules, the algorithm will also gain a breadth of knowledge. We believe using deep learning algorithms will be particularly beneficial in the evaluation of nodules of indeterminate cytology and could decrease the need for diagnostic excision.

437 Cytologic Rapid On-Site Evaluation (ROSE) and Flow Cytometric Analysis in the Needle Biopsy Diagnosis of Thymoma

Liye Suo[1], Yimin Ge[2], Roberto Barrios[3], Mary Schwartz[2], Michael Thrall[2], Haijun (Steve) Zhou[2]
[1]Baylor College of Medicine, Houston, TX, [2]Houston Methodist Hospital, Houston, TX, [3]The Methodist Hospital, Houston, TX

Disclosures: Liye Suo: None; Roberto Barrios: None; Mary Schwartz: None; Michael Thrall: None; Haijun (Steve) Zhou: None

Background: Thymoma is the most common primary malignancy of the anterior mediastinum. The variable amounts of the biphasic epithelial and lymphoid components in cytologic samples pose diagnostic pitfalls, and can lead to incorrect interpretation of thymomas as either lymphoid lesions or epithelioid malignancies.

Design: We searched our pathology database between 2014 and 2019 for cytology rapid on-site evaluation (ROSE) of cases that were histologically confirmed to be thymoma. We retrospectively reviewed the diagnostic accuracy of cytologic evaluation, and the use of flow cytometry as an effective adjunct diagnostic modality.

Results: Eighteen thymic tumor cases with cytology rapid on-site evaluation were identified. Fourteen cases had epithelioid/spindled cells, as well as lymphoid elements present, which enabled the cytologic diagnosis of thymoma. For three cases with only a lymphoid component appreciated on cytologic evaluation, thymocytes were identified by flow cytometry and lymphoma was ruled out. A total of sixteen cases were submitted for flow cytometric analysis and thymocytes were identified in thirteen of these cases. For those three cases without thymocytes identified on flow cytometry, cytologic evaluation showed adequate specimen for diagnosis of thymoma with biphasic components in two cases. One case with only epithelial cells seen on cytologic evaluation and only peripheral circulating lymphocytes found on flow cytometry was demonstrated on tissue histologic examination to be a type B3 thymoma.

Conclusions: Cytologic rapid on-site evaluation of thymoma can be challenging when the classic biphasic components are not apparent. Triaging samples for flow cytometry analysis increases the diagnostic accuracy of cytology evaluation when only a lymphoid component is cytologically appreciated. For cases with only an epithelioid component seen on cytologic ROSE, adequate tissue procurement for additional studies is important.

438 Urine Cytology Diagnostic Patterns Before and After Implementing the Paris System for Reporting Urine Cytology: A Large Single Institutional Study of Over 27,000 Cases

Wei Tian[1], Karen Shore[2], Rajal Shah[3]
[1]Inform Diagnostics, Irving, TX, [2]Rice University, Houston, TX, [3]University of Texas Southwestern Medical Center, Plano, TX

Disclosures: Wei Tian: None; Karen Shore: None; Rajal Shah: None

Background: Management of urothelial carcinoma (UC) is expensive due to a long disease course. Urine cytology is a noninvasive and cost-effective diagnostic and surveillance method in clinical management of UC. The Paris System for Reporting Urine Cytology (TPS) was published in 2016, and introduced more definite diagnostic criteria aimed to improve sensitivity and specificity in detecting high grade UC (HGUC).

Design: We performed a retrospective review of urine cytology reported in two years from Jan. 2013 to Dec. 2014 (prior to TPS, group 1, total of 7,659 cases), and two years from May 2016 to April 2018 (after TPS, group 2, total of 20,027 cases) to assess the influence of TPS in our practice. Cases with insufficient cellularity were excluded. Time between Jan. 2015 to April 2016 was considered a learning period. We implemented initial grand rounds presentation followed by biweekly urine cytology conferences for difficulty cases to practice the utilization of the diagnostic criteria in TPS. The comparison was made between the two groups in diagnostic categories, and UroVysion FISH correlation when available.

Results: Urine cytology diagnoses prior to TPS included negative for UC (NUC) 5,294 (69%), atypical urothelial cells (AUC) 2,227 (29%) (23-39% among pathologists), including mildly atypical favor reactive changes (MAUC) 1,437 (18.8%), and suspicious or positive for HGUC (SHGUC/HGUC) 138 (1.8%). Diagnoses after TPS included negative for HGUC (NHGUC/NUC) 18,507 (92.4%), AUC 1,238 (6.2%) (5.3-7.2% among pathologists) (including MAUC 392 cases, 2%), and SHGUC/HGUC 282 (1.4%). Comparing groups 1 and 2 (Table 1), AUC dropped from 29% to 6.2% ($p<0.00001$), among which MAUC decreased from 18.8% to 2%. All GU pathologists showed significant decrease in AUC ($P<0.00001$, data not shown). Due to lack of follow-up bladder biopsies in our practice, we reviewed UroVysion FISH results in AUC. Prior to TPS, 980/2,227 (44%) AUC had FISH results and 164 (16.7%) were positive. After TPS, 550/1,238 (44.4%) had FISH results, and 207 (37.6%) were positive ($p<0.00001$).

Table 1. Urine cytology diagnostic categories before and after TPS.

Diagnosis	Group 1		Group 2		P Value
	n	%	n	%	
NUC/NHGUC	5,294	69.2%	18,507	92.4%	<0.00001
AUC	2,227	29%	1,238	6.2%	<0.00001
(MAUC)	(1,437)	(18.8%)	(392)	(2%)	<0.00001
SHGUC/HGUC	138	1.8%	282	1.4%	0.0057
Total	7,659	100%	20,027	100%	

Conclusions: Implementing TPS resulted in a significant decrease in urine cytology atypical diagnoses from 29% to 6.2%. Most noticeably, the MAUC was dramatically decreased from 18.8% to 2%, even though we had not completely eliminated it. In addition, after TPS, AUC was significantly better correlated with UroVysion FISH results, which decreased the number of FISH tests and saved medical costs.

439 Endobronchial Ultrasound-Guided Fine Needle Aspiration (EBUS-FNA): A Powerful Modality to Obtain Adequate Samples for PD-L1 Expression

Kevin Trowell[1], Khurram Shafique[2], Cindy McGrath[1], Darshana Jhala[3]
[1]Hospital of the University of Pennsylvania, Philadelphia, PA, [2]Fargo, ND, [3]Philadelphia VA Medical Center and University of Pennsylvania, Bryn Mawr, PA

Disclosures: Kevin Trowell: None; Khurram Shafique: None; Cindy McGrath: None; Darshana Jhala: None

Background: Evaluation of PD-L1 expression by immunohistochemistry (IHC) is recommended for non-small cell lung carcinoma (NSCLC) to determine eligibility for anti PD-L1 immunotherapy. Endobronchial ultrasound-guided fine needle aspiration (EBUS-FNA) has been

proven to be highly useful in obtaining material for diagnosis, staging, and ancillary studies in lung cancer. At our institution, EBUS-FNA samples that are positive for non-small cell lung carcinomas are routinely evaluated for adequacy for ancillary testing. As a part of a quality assurance project, we reviewed cytology specimens obtained by EBUS-FNA that had PD-L1 IHC performed.

Design: At a major tertiary center, we examined all EBUS-FNA cell block specimens which had PD-L1 IHC (22C3 antibody) performed at our institution from January 2017 to August 2017. A minimum of 100 viable tumor cells must be present to evaluate PD-L1 IHC staining, and partial or complete membranous staining in viable tumor cells is considered positive staining. Results are reported as negative (<1% cells), positive – low expression (1-49%), and positive - high expression (≥50%). While surgical specimens are preferred for PD-L1 IHC, cell blocks can be utilized when adequate.

Results: From January 2017 to August 2017, PD-L1 IHC was performed on 89 EBUS-FNA cell blocks. The results are shown in the provided table. Diagnoses rendered were as follows: adenocarcinoma (n=60), squamous cell carcinoma (n=13), adenosquamous carcinoma (n=2), NSCLC-not otherwise specified (n=10). Four cases were from non-NSCLC tumors, which included two cases of small cell carcinoma and two cases of metastatic tumors. All cases were assessed for tumor cellularity and deemed adequate for ancillary immunohistochemistry studies at the time PD-L1 IHC was ordered. Two (2.2%) cases had PD-L1 IHC performed, but were ultimately inadequate for evaluation.

Thirteen cases had concurrent surgical biopsies. In 4/13 cases, both surgical biopsy and EBUS-FNA specimens contained adequate material for ancillary testing. In the remaining 9/13 cases, the surgical biopsy specimen was either negative or inadequate, while the cytology specimen was adequate.

Total number of cases	89
Diagnosis on cytology - no. (%)	
Adenocarcinoma	60 (67.4%)
Squamous cell carcinoma	13 (14.6%)
Adenosquamous carcinoma	2 (2.2%)
NSCLC, NOS	10 (11.2%)
Other	4 (4.5%)
PD-L1 results	
Negative (<1%)	45 (50.5%)
Low Positive (1-50%)	29 (32.6%)
High Positive (>50%)	13 (14.6%)
Inadequate	2 (2.2%)
Cases with concurrent surgical biopsy	13
Biopsy and cytology adequate for PD-L1	4 (30.8%)
Biopsy inadequate, cytology adequate for PD-L1	6 (46.2%)
Biopsy negative, cytology adequate for PD-L1	3 (23.1%)

Figure 1 - 439

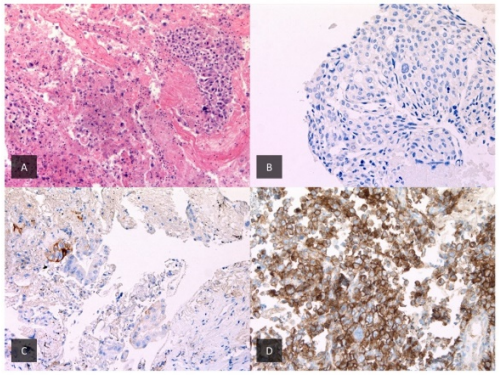


Figure 1: Representative cytology cell block showing carcinoma with non-small cell morphology, H&E (A); representative cases of PD-L1 IHC performed on cytology cell blocks showing no expression (B), low expression (C), and high expression (D).

Conclusions: EBUS-FNA cell block specimens are an important tool for the assessment of PD-L1 expression in lung cancer:

- PD-L1 IHC can be reliably performed on adequacy-assessed EBUS-FNA cell block specimens
- In 9/13 cases of concurrently obtained EBUS-FNA and surgical biopsies, EBUS-FNA cell blocks were relied upon for PD-L1 IHC

440 Fine Needle Aspiration and Core Needle Biopsy Have Similar Diagnostic Performance for Fibroepithelial Lesions of Breast

Kent Truong[1], Dianna Ng[2], Frances Castillo-Llanda[2], Poonam Vohra[1]
[1]University of California San Francisco, San Francisco, CA, [2]University of California San Francisco Medical Center, San Francisco, CA

Disclosures: Kent Truong: None; Dianna Ng: *Primary Investigator*, Cepheid, Inc.; Frances Castillo-Llanda: None; Poonam Vohra: None

Background: Fibroepithelial lesions (FEL), including fibroadenoma (FA) and phyllodes tumor (PT), are challenging to diagnose on fine needle aspiration (FNA) and core needle biopsy (CNB) due to overlapping cytologic and architectural features. This study aims to evaluate our institutional performance of FNA and corresponding CNB in diagnosing FELs.

Design: We identified all FNAs between 2003-2016 with a diagnosis of FA, FEL, or PT and correlated them with any follow-up core needle biopsy (CNB) or surgical excision (SE) diagnoses. Diagnoses were categorized as benign, indeterminate or malignant. Clinical data, including patient age, mass size and BI-RADS score was collected.

Results: 775 FNAs were identified, of which 26 had both CNB and SE, 66 SE only and 64 CNB only. Median age was 38 years old (range: 13-78 years old) and median mass size was 1.5 cm (range: 0.12 – 14 cm). All PTs by SE had a BI-RADS score of 4 and above (p=0.01). FNA and CNB diagnosed as indeterminate were larger compared to benign (1.5 vs 2.2 cm, p=0.005). Of FNA, 748 were FA (97%), 24 indeterminate (3%) and 3 PT (<1%). Of the 748 FA by FNA, 77 received SE, of which 52 were FA (67%), 15 benign phyllodes tumor (BPT) (20%), 1 MPT (1%), 6 OB (8%), and 3 invasive ductal carcinoma (IDC) (4%). If FA on FNA cases without SE are assumed to be FAs, then 15/748 (2%) BPTs and 1/748 (0.1%) MPTs were misclassified. There were 21 FEL by FNA, with 3 FA (14%), 6 BPT (29%), and 2 MPT (9%) on SE, while 10 (48%) had no SE. Three cases were PT on FNA, with 1 BPT and 1 leiomyosarcoma on SE while 1 had no SE. Of CNB, there were 38 FA (43%), 19 FEL (21%), 3 IDC (3%), 4 BPT (5%), 6 PT (7%), 17 other benign (OB) (19%) and 2 ductal carcinoma in situ (2%). Of the 38 FA on CNB, one was BPT (3%) and 3 FA (8%) on SE while 34 had no SE (89%). Of the 19 FEL on CNB, 2 were FA, 7 were BPT, and 2 MPT on SE while 8 had no SE. For lesions called FA kappa agreement with SE was 0.09 for FNA and -0.01 for CNB.

Conclusions: FNA and CNB have similar kappa agreement with the SE diagnosis, and a clear cut distinction between FA and benign PT is often not possible either by FNA or CNB. CNB after a diagnosis of FEL on FNA is not necessary and does not provide a more definitive diagnosis. Therefore it is prudent to excise FEL on FNA to prevent false negative diagnoses. Incidence of any PT is low (2%) and of MPT is rare (0.1%) subsequent to a diagnosis of FA on FNA. Diagnosing FA on FNA is reliable and safe, provided there is adequate imaging correlation and follow up.

441 Digital Pathology for Cell Block Evaluation, Does It Work?

Kent Truong[1], Sarah Calkins[1], Tara Saunders[1], Elham Khanafshar[1]
[1]*University of California San Francisco, San Francisco, CA*

Disclosures: Kent Truong: None; Sarah Calkins: None; Tara Saunders: None; Elham Khanafshar: None

Background: Digital pathology for primary diagnosis is a rapidly developing field. Many studies address the digital image (DI) quality of histologic slides and aspirate smears, but few have evaluated the DI quality of cytology cell block (CB) material. This study aims to compare the quality of DIs of CB material to original glass slides (GS).

Design: 50 consecutive cytology cases with CB material were retrieved from file. All CBs were made using the collodion bag technique. A single hematoxylin and eosin stained glass slide with 2 or 3 sections was prepared for each CB.
Of the 50 cases, 46 had a single CB and 4 had two CBs for a total of 54 GS for evaluation. Of these, 35 were from fine needle aspiration specimens from various sources and 19 were from various body fluids.
GS were digitally scanned per manufacturer instructions using a Philips Ultra Fast Scanner (UFS).
Four board-certified pathologists compared each DI to the corresponding GS, documenting quality and completeness of scan.

Results: All 50 cases (54 GS) contained material, ranging from sparsely to abundantly cellular. Material was present on the DI for 50/54 (93%) scanned GS. No material was scanned for 4 GS despite the presence of rare cells and visible collodion bags (fig. 1).
Of the DI which showed scanned material, 41/50 (82%) showed equivalent cytologic quality to the GS (fig. 1). Of these, 16/41 (39%) were missing some degree of material present on the GS (fig. 1). In four GS with multiple similar appearing sections, the UFS showed significant inconsistency between sections in identifying the presence of material (fig. 2).
Of the 9 DI with suboptimal quality, 7 (78%) had blurry DI quality at 40x magnification, 1 had an entire section grossly out of focus, and 1 showed significant blurriness due to tissue folding (Z-plane focus).

Figure 1 - 441

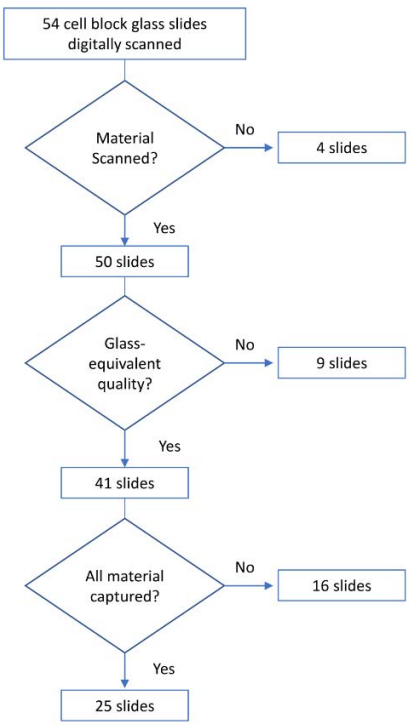
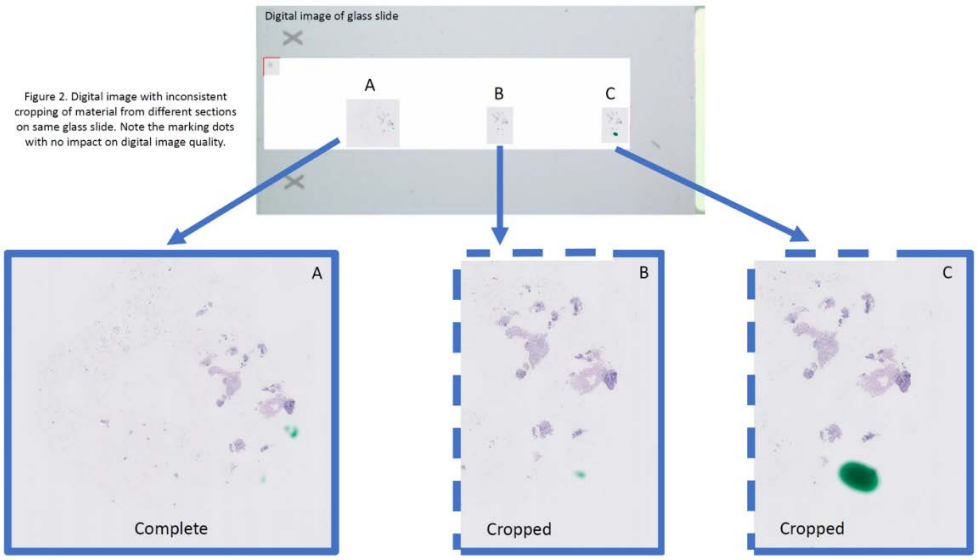


Figure 1. Summary of cell block digital image quality and completeness.

Figure 2 - 441



Conclusions: In our study, 25/54 (46%) digitally scanned CBs were scanned completely and with quality equivalent to GS. In the suboptimally scanned CBs, inconsistent scanning of material and blurry DI quality at 40x were notable problems. Despite some DI having blurry quality at 40x, they would all allow for triage for additional studies such as immunohistochemistry and potentially aid in turnaround time, especially if using an off-site Histology Lab. Further studies are required to optimize the digital scan quality of CB material for routine clinical use.

442 Anal Cytology and High-RiskHPV Testing: A Retrospective Study with Histologic Follow-up

Aram Vosoughi[1], Adebowale Adeniran[2], Guoping Cai[3], Rita Abi-Raad[1]
[1]Yale University School of Medicine, New Haven, CT, [2]Yale University School of Medicine, Seymour, CT, [3]Yale University, Wallingford, CT

Disclosures: Aram Vosoughi: None; Adebowale Adeniran: None; Guoping Cai: None; Rita Abi-Raad: None

Background: In high-risk populations, such as HIV positive patients and men who have sex with men, anal cytology screening (ACS) is advocated for surveillance and detection of anal precancerous lesions or anal intraepithelial neoplasia (AIN). However, the potential role of ACS in preventing anal cancer is debatable. Furthermore, the value of high-risk HPV (hrHPV) testing is not well established. Here, we review our experience with ACS and hrHPV testing in conjunction with histologic outcomes.

Design: The departmental cytology database was searched for all ACS performed between January 1, 2016 and December 31, 2018. An abnormal ACS was defined as any abnormal cytology diagnosis (ASCUS and above). Concurrent hrHPV result, if available, and subsequent pertinent anal biopsy results were recorded.

Results: A total of 795 ACS were performed and the diagnosis included: unsatisfactory (15%), NILM (53%), ASCUS (21%), LSIL (9%), ASC-H (1%) and HSIL (1%). Follow-up biopsy was available in 93 cases (Table 1). 64 (69%) patients were men and 29 (31%) were women with a median age of 51 years. The overall risk for AIN2+ in patients with an abnormal ACS was 47%. The risk of AIN2+ was 86% in patients with ASC-H or HSIL and 43% in those with ASCUS or LSIL. The sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) of an abnormal ACS in detecting AIN2+ lesions were 91%, 35%, 48% and 86%, respectively.

hrHPV result was available in 46 cases (36 positive and 10 negative) with diagnoses of NILM (9 cases), ASCUS (24 cases), LSIL (10 cases) and ASC-H (3 cases). Of positive hrHPV patients, 47% (17/36) had AIN2+ on biopsy. All but one patient had an abnormal ACS (16/17; 94%). Of negative hrHPV, 2 out of 10 patients (20%) had AIN2+ on biopsy; both patients had ASCUS diagnosis on cytology. With hrHPV testing, the performance of an abnormal ACS in detecting AIN2+ was as follows: sensitivity = 89%, specificity = 26%, PPV = 53% and NPV = 71%. Interestingly, of 22 NILM cases, 3 patients had AIN2+ on biopsy: hrHPV was positive in one patient and unavailable in the two others, suggesting that hrHPV testing might improve AIN2+ detection in NILM anal cytology.

CYTOLOGY	CASE (n)	BIOPSY n (%)		
		BENIGN	AIN1	AIN2-3
UNSAT	4	3 (75)	1 (25)	0 (0)
NILM	22	13 (59)	6 (27)	3 (14)
ASCUS	39	14 (36)	9 (23)	16 (41)
LSIL	21	4 (19)	7 (33)	10 (48)
ASC-H	3	0 (0)	0 (0)	3 (100)
HSIL	4	1 (25)	0 (0)	3 (75)

Conclusions: Regardless of hrHPV results, ACS is highly sensitive but not specific for the detection of AIN2+. In our study, hrHPV testing does not seem to enhance ACS performance but might be beneficial in NILM cases. Our findings underscore the benefit of cotesting in anal cytology.

443 Cervical Malignancies with Negative High Risk HPV: Why Cervical Cytology is Being Retained at One Academic Institution

Saloni Walia[1], Pamela Ward[2], Lan Du[2], Tiffany Long[2], Louis Dubeau[3], Wesley Naritoku[2], Sue Martin[2]
[1]Keck School of Medicine of University of Southern California, Manhattan Beach, CA, [2]Keck School of Medicine of University of Southern California, Los Angeles, CA, [3]University of Southern California, Los Angeles, CA

Disclosures: Saloni Walia: None; Pamela Ward: *Advisory Board Member*, Roche Molecular Diagnostics; Lan Du: None; Tiffany Long: None; Louis Dubeau: None; Wesley Naritoku: None; Sue Martin: None

Background: Cervical screening by cytology alone has shown remarkable success in decreasing the incidence of cervical cancer by up to 80%. Since cervical cancers are primarily caused by human papillomavirus (HPV), the FDA has approved the Roche cobas® HPV test for primary screening. However, screening with the cobas test for 14 high risk subtypes of HPV (HR-HPV) alone has the potential for false negative results in cases where infection is due to other HPV subtypes or in malignancies unrelated to HPV. False negative HPV tests are significant because missing early detection can lead to a low 5-year survival rate in patients who develop metastatic disease. The aim of this study was to use the results of HPV co-testing and reflex testing to predict the efficacy of HPV primary screening in a tertiary care hospital.

Design: In this retrospective study, data from cervical specimens that underwent both cytology and HPV testing were collected and analyzed. The cytology was performed on ThinPrep® Pap test, while PCR-based testing was performed using the Roche cobas® HPV test.

Results: Of 8190 samples, 564 cases were positive by both cytology and HR-HPV testing. A total of 826 (10.1%) cases were cytology negative but HR-HPV positive. Within this cytology negative and HR-HPV positive group, 170 cases were HPV-16 and/or -18 positive. Follow-up was available for 128 cases, of which 19 showed a high grade squamous intraepithelial lesion (HSIL). No invasive carcinoma was identified in this cohort.

Of 6800 HR-HPV negative cases, 381 (5.6%) showed epithelial abnormalities by cytology (atypical glandular cells, atypical squamous cells of undetermined significance, or higher). Twenty-seven of these cases had a clinically actionable cytologic diagnosis (either atypical glandular, atypical squamous cells – cannot rule out high grade, HSIL, or invasive carcinoma). In the 26 cases with follow up, 2 patients had endocervical adenocarcinomas; 1, squamous cell carcinoma; 1, HSIL; 1, anal carcinoma; and 6, endometrial adenocarcinoma.

Conclusions: Our findings would predict that primary HPV screening alone would have led to a delay in detection of 3 cervical carcinomas and 1 HSIL out of 6800 HR-HPV negative cases. These findings suggest that cervical cytology with HPV co- testing and reflex testing, should be used as best practice for screening cervical cancer.

444 Revealing the P16 Positivity Thresholds in Cytology Cell Blocks of Oropharyngeal Squamous Cell Carcinoma- A Comparison with Surgical Pathology P16 Staining

Qian Wang[1], Justin Snow[2], Aylin Simsir[3], Pascale Levine[4], Oliver Szeto[5], Wei Sun[6], Osvaldo Hernandez[7], Tamar Brandler[8]
[1]NYU Langone Health, New York City, NY, [2]New York University Langone Health, New York, NY, [3]New York University School of Medicine, Edison, NJ, [4]New York University Medical Center, New York, NY, [5]NYU Langone Health, New York, NY, [6]New York University Langone Medical Center, New York, NY, [7]New York, NY, [8]NYU Langone Health, Highland Park, NJ

Disclosures: Qian Wang: None; Justin Snow: None; Aylin Simsir: None; Pascale Levine: None; Oliver Szeto: None; Wei Sun: None; Osvaldo Hernandez: None; Tamar Brandler: None

Background: HPV-related oropharyngeal squamous cell carcinoma (OP-SCC) has a superior prognosis and response to therapy than that of conventional head-and neck SCC (HNSCC). The College of American Pathologists (CAP) guidelines recommend that P16 immunostaining (IHC) in >70% of tumor cells is an excellent surrogate marker for HPV in surgical pathology OP-SCC. Fine needle aspiration (FNA) cytology is an ideal method for obtaining diagnostic material for OP-SCC and may represent the only attainable specimen. However, there is no consensus for interpretation of P16 IHC result in cytology preparations. Our study aims to assess OP-SCC P16 staining in cell block cytology preparations in comparison with P16 staining on surgical pathology specimens.

Design: FNA specimens from 2014-2019 of OP-SCC with P16 IHC staining were obtained. Surgical pathology P16 IHC results were set as the gold standard. Cytology cell block tumor cellularity (<100 vs >100 cells) and P16 percentage of tumor cell positivity (0%, 1-10%, 11-50%, 51-70%, and >70%) were recorded. Using different threshold levels of P16 tumor cell positivity in cell blocks as compared with surgical P16 IHC results, sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) were calculated.

Results: 40 matched FNA neck lymph node/mass cytology and surgical cases were identified. Sensitivities and specificities varied when thresholds changed, with sensitivities and specificities ranging from 93.5% and 66.7% (respectively) when any P16 positivity is seen (>0%), to 56.7% and 100% (respectively) when P16 positive threshold is set at >70% (table 1 and figure 1). <100 and >100 tumor cells were seen in 11 and 29 cases respectively.

Table 1. Correlation of P16 positivity between cytology cell block and surgical cases using different P16 positivity thresholds in cell block

threshold for p16 in cytology		surgical p16 + (case number)	surgical p16- (case number)	total number	sensitivity	specificity	PPV	NPV
≥1%	cytology p16+	29	3	32	94%	67%	91%	75%
	cytology p16-	2	6	8				
>10%	cytology p16+	22	1	23	71%	89%	96%	47%
	cytology p16-	9	8	17				
>50%	cytology p16+	18	1	19	60%	90%	95%	43%
	cytology p16-	12	9	21				
>70%	cytology p16+	17	0	17	57%	100%	100%	43%
	cytology p16-	13	10	23				

Figure 1 - 444

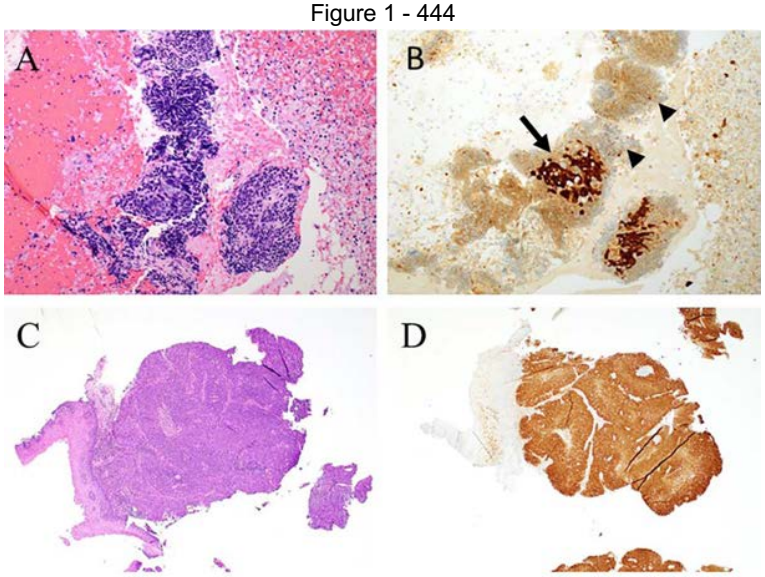


Figure 1. Cytology cell block shows OP-SCC (A. Hematoxylin and eosin, 200x) with nuclear and cytoplasmic, "block-like" positive P16 immunostaining (arrow) and negative, non-specific staining (arrow head) (B. 200x). C. Surgical OP-SCC biopsy (Hematoxylin and eosin, 40x) with "block-like" nuclear and cytoplasmic P16 staining (D. 40x).

Conclusions: Our study shows that P16 IHC performed on cytology cell blocks can serve as a surrogate marker for the detection of HPV, similar to P16 staining in surgical pathology, with high sensitivity and specificity levels. The challenge in cytology specimens is choosing the proper threshold to balance between the optimal sensitivity and specificity. Our data suggests that using a threshold lower than that of surgical pathology (70%) for p16 positivity may be appropriate for FNA specimens, as lower thresholds displayed increased sensitivities with only moderately lower specificities. Of note out of the 11 cases with <100 tumor cells, only one cases was a false negative, indicating that tumor cellularity may not affect P16 interpretation on cell block.

445 Molecular Testing for ROS1 Rearrangements and Morphological Characterization in the Cytological and Limited Biopsy Specimens from the Patients with Non-Small Cell Lung Cancers

Xiaoyan Wang[1], Shaobo Zhang[2], Minaxiben Patel[3], Liang Cheng[2]
[1]Indiana University, Pathology & Laboratory Medicine, Indianapolis, IN, [2]Indiana University School of Medicine, Indianapolis, IN, [3]RLR VA Medical Center, Indianapolis, IN

Disclosures: Xiaoyan Wang: None; Shaobo Zhang: None; Minaxiben Patel: None; Liang Cheng: None

Background: Identifying for ROS proto-oncogene 1 receptor tryrosine kinase (ROS1) fusion rearrangement in lung cancers has become increasingly important because of successful targeted therapies among a subset of patients without other oncogenic mutations. Cytology remains the initial approach in establishing the diagnosis of lung cancers due to the minimally invasive nature of procedure. This study is undertaken to investigate ROS1 rearrangement by fluorescence in situ hybridization (FISH) using cytological and limited biopsy specimens from the patients with primary or metastatic lung cancers and to further characterize the cytomorphologic features of these tumors.

Design: Patients with primary or metastatic non-small cell lung carcinomas tested for ROS1 rearrangement were retrospectively identified from January 2014 to June 2019. A total 1452 cases were submitted for ROS1 rearrangement analysis. The specimen sources included lung, lymph node, liver, bone, adrenal gland, mesentery mass, and body fluids. FISH studies for ROS1 rearrangement 6q22 were performed on the paraffin embedded cell block or limited biopsy sections utilizing dual fluorescence color-labeled ROS1 break-apart FISH probes (Vysis, Downers Grove, IL). Diff-Quik and Papanicolaou-stained smears as well as the cell block/limited biopsy sections were also examined cytomorphologically.

Results: Among 1452 cases submitted for analysis, 35 cases were positive for ROS1 rearrangement (2.4%). Twenty nine out of 35 (83%) ROS1 positive cases were cytological or limited biopsy samples. Cytomorphologically, smears from the ROS1 positive cases often displayed three dimensional cohesive cell groups with variable amount of cytoplasm, pleomorphic nuclei, irregular nuclear contour, and prominent nucleoli. The cell block or limited biopsy samples exhibited tumor cells in acinar, solid, lepidic, less commonly papillary/micropapillary growth pattern with frequently eosinophilic cytoplasm, and rarely with signet ring cell features.

Conclusions: The current study demonstrates that cytological or limited biopsy specimens can yield sufficient material for ROS1 rearrangement test. Our study reveals 2.4% of ROS1 rearrangement rate in the patients with lung non-small cell carcinomas. In addition, ROS1 rearrangement may be useful to predict certain cytomorphologic features of lung cancers.

446 Utility of Ampullary Brushings and Endoscopic-Guided Fine Needle Aspiration Cytology in Preoperative Assessment and Management of Tumors of Ampulla of Vater

Michael Williams[1], Muhammad Arif[1], Kamal Khurana[2]
[1]SUNY Upstate Medical University, Syracuse, NY, [2]SUNY Health Science Center, Syracuse, NY

Disclosures: Michael Williams: None; Muhammad Arif: None; Kamal Khurana: None

Background: The utility of ampullary brushing cytology and endoscopic guided fine needle aspiration (EUS-FNA) cytology in preoperative assessment and management of intra-ampullary tumors is not well documented in the literature. We assessed the diagnostic accuracy of cytology and analyzed the causes of discrepancy among clinical, histologic and cytologic parameters in the largest series of cases with ampullary lesions.

Design: A retrospective electronic database review was performed from the years 2008 to 2019 to identify patients who had ampullary brushings or EUS-FNA for suspected ampullary neoplasms. Cases with available clinical or histologic follow-up were reviewed. All negative cases had a biopsy follow up or at least one year of clinical follow up. Standard interpretative categories- negative, atypical, suspicious, and positive-were utilized for preoperative cytology specimens. Atypical/suspicious category were combined with positive category for calculation of sensitivity, specificity, positive and negative predictive values.

Results: A total of 39 Ampullary FNAs (26) and brushings (13) were identified. The mean age was 70 years with M:F ratio 0.7:1. The sensitivity, specificity, positive predictive value and negative predictive value were 78.5%, 88%, 78.7 and 88%, respectively. Three false positive cases with 'atypical' cytology interpretation revealed thermal artefact (1 case), hyperplastic polyp (1 case) and paraganglioma (1 case). Three false negative cases were attributed to cyto-sampling. Eleven cases with negative cytology did not show any progression of lesion on clinical follow up for at least one year. Histologic and clinical follow up yielded a diagnostic concordance of 84.8%.

Conclusions: Ampullary brushings and EUS-FNA are useful diagnostic modalities for preoperative assessment and management of tumors of ampulla of Vater and has a high diagnostic accuracy.

447 HER2 Testing in Effusion Samples: Concordance with Tissue Samples

Yubo Wu[1], Athena Chen[1], Paul VanderLaan[1], Liza Quintana[1]
[1]Beth Israel Deaconess Medical Center, Boston, MA

Disclosures: Yubo Wu: None; Athena Chen: None; Paul VanderLaan: None; Liza Quintana: None

Background: Molecular testing of pathology specimens is increasingly important for providing diagnostic, prognostic, and predictive information. HER2, routinely used in breast, gastrointestinal, and now gynecologic pathology, is one such biomarker. Validation and reporting guidelines for HER2 testing are based on formalin-fixed paraffin embedded (FFPE) tissue, with minimal recommendations for cytopathology samples. Most of the published literature shows good concordance between FFPE and cytopathology samples; however, evaluation has been performed largely on aspirated material from solid masses. We reviewed our institutional experience with HER2 expression by tumor cells in metastases to effusion fluids.

Design: Effusion specimens with HER2 performed at our institution on cell blocks (CB) from 1/2017-12/2018 were identified. All CBs were FFPE. HER2 was compared to prior/concurrent tissue block (TB) HER2 results. We reviewed slides from all discordant cases.

Results: 20 cases had CB and TB HER2 results. 18 patients were female and 1 male; the median age was 67 (range 35-85). Samples were 14 pleural, 5 peritoneal/ascites, and 1 pericardial fluid. Median fluid volume was 575 mL (range 5-1000 mL). Metastases were from 17 breast, 1 gastric, and 1 esophageal primary tumors. Clinical HER2 status between TB and CB are summarized in Table 1. 18 (90%) of cases had concordant HER2 results. The 2 (10%) discordant cases were 1 esophageal and 1 breast malignancies. At the time of diagnosis, the esophageal carcinoma metastasis was noted to have different morphology in the CB compared to the TB/primary tumor. The discordant breast carcinoma was estrogen (ER) and progesterone receptor (PR) positive and HER2 negative by FISH in 2006, however, the subsequent pleural fluid metastasis in 2018 was ER and PR negative and HER2 positive.

HER2 Tissue Block	HER2 Cell Block	
	Positive	Negative
Positive	4	1
Negative	1	14

Conclusions: HER2 status contributes to management of breast and increasingly subtypes of gastrointestinal and gynecologic malignancies. As such, it is important to ensure that the substrate on which HER2 testing is done is evaluable. In our cohort, we found excellent concordance between clinical HER2 status in CB and TB preparations. Our 2 discordant cases consisted of a metastatic esophageal carcinoma with dissimilar morphology compared to the prior and a breast carcinoma with completely different hormone receptor and HER2 profile, suggesting clonal evolution or an undiagnosed second primary carcinoma. Our data support the use of effusion samples for HER2 testing.

448 Atypical Cerebrospinal Fluid Cytology Findings in Chimeric Antigen Receptor T-cell Immunotherapy

Helmae Wubneh[1], Mark Roth[2], Armando Filie[2], Nirali Shah[1], Hao-Wei Wang[3]
[1]National Cancer Institute, National Institutes of Health, Bethesda, MD, [2]National Institutes of Health/National Cancer Institute, Bethesda, MD, [3]Bethesda, MD

Disclosures: Helmae Wubneh: None; Armando Filie: None; Hao-Wei Wang: None

Background: Chimeric antigen receptor T (CAR-T)-cell immunotherapy clinical trials in the treatment of relapsed and/or refractory +CD19 or +CD22 hematologic malignancies have shown significant promise, altering clinical endpoints especially in patients who have failed chemotherapeutic regimens and/or stem cell transplant. In this study, we evaluate the atypical cytologic findings of CSF specimens from patients who have received CAR-T cell therapy.

Design: We identified CSF specimens from clinical trial patients undergoing CAR-T cell therapy for B-cell lineage leukemias and lymphomas between 2012-2019 that showed atypical changes not morphologically diagnostic of involvement by disease. The slides were retrieved from our files in order to further characterize the atypical changes. Clinicopathologic correlation with the prior presence of CNS disease as well as documentation of flow cytometric findings to include the quantitative assessment of CAR-T cells were also performed.

Results: The atypical cytologic findings include the presence of atypical lymphoid/mononuclear cells seen in 11 patients (21 cases). Examination of these 21 CSF cases revealed a range of cytologic atypical features including the presence of blasts (5cases; 24%) and atypical lymphoid/mononuclear cells other than blasts (18 cases, 85%). These atypical lymphoid/mononuclear cells were small (5 cases), small to medium (6 cases) and medium to large (7 cases) in size; with either mild (10 cases), moderate (7 cases) or marked (1 case) nuclear atypia. Of note, 1 atypical CSF specimen revealed scattered large markedly atypical lymphoid cells in a background of a polymorphous population of lymphocytes (Figure 1), but no immunophenotypic evidence of an abnormal B cell population by flow cytometry; interestingly, +CD22 chimeric T cells represent 85% of T cells present in the specimen.

Figure 1 - 448

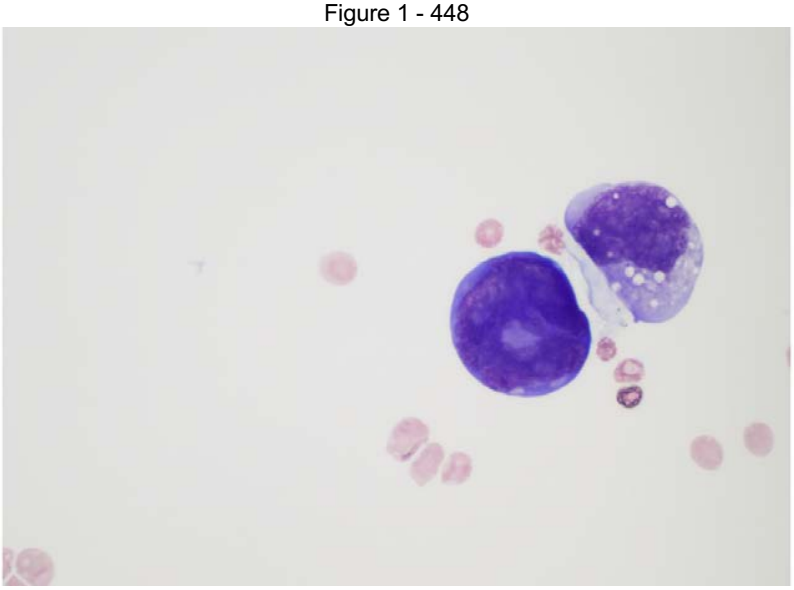


Figure 1

Conclusions: With the increasing use of CAR-T cell therapy, pathologists should be more familiar with the cytomorphologic spectrum of findings in CSF samples of patients undergoing CAR-T cell therapy for relapsed and/or refractory hematologic malignancies. The spectrum of cytologic findings in this patient population includes the presence of small atypical lymphoid/mononuclear cells with mild nuclear atypia to large cells with marked nuclear atypia; therefore, close correlation with flow cytometry may be needed to distinguish blasts from CAR-T cells.

449 Measuring the Utility of Telecytology in Rapid Onsite Evaluation: Preliminary Diagnosis and Adequacy of Lymph Nodes Cytology Collected by EUS and EBUS Guided Needle Aspiration

Hira Yousaf[1], Khalid Amin[1], Tetyana Mettler[2], Jimmie Stewart[1]
[1]University of Minnesota, Minneapolis, MN, [2]University of Minnesota, Chanhassen, MN

Disclosures: Hira Yousaf: None; Khalid Amin: None; Tetyana Mettler: None; Jimmie Stewart: None

Background: Dynamic telecytopathology is frequently used for rapid on site evaluation (ROSE). It aids in adequacy assessments and in preliminary diagnoses for patient triage. A prospective study was performed to validate telecytopathology in providing preliminary diagnoses and ROSE for lymph node cases in EUS and EBUS.

Design: Specimens from lymph nodes through EUS and EBUS procedures were gathered from 6/2018-12/2018. A numerical scoring system was established, ranging from 0-4. 0-non diagnostic (ND), 1- Negative, 2-Atypical, 3- Suspicious and 4-Positive. Using this scheme, each case was graded on telecytopathology review (TR), followed by a microscopic review (MR) of the same slide by the same pathologist and later on final diagnosis (FD). Concordance rates (CR) between TR, MR and FD were calculated for major discrepancy (MD) as it could represent possible clinical management interference. MD was defined as a difference of two categories after revaluation.

Results: There were 195 cases: 132 – EBUS and 63 - EUS procedures. 5 cases from EBUS had MD between TR and FD, and 6 from EUS with a total of 11 cases. For TR vs. MR, EBUS had 2 cases with MD and EUS had 1, with a total of 3 cases. Among the TR vs FD discrepant cases, 2 from EUS and 1from EBUS were lymphoma diagnoses. 10 of 11 discrepant cases (90.9%) were under call on TR (all 5 from EBUS and 5/6 from EUS). 14 specimens were non-diagnostic (ND) on FD, all from the EBUS procedures making a ND rate of 7.18%, 0% and 10.6%, for overall, EUS and EBUS respectively. Overall CR between TR and FD is 94.3% and TR and MR is 98.5%. CR for EBUS is 96.2% and 98.5% for TR vs. FD and TR vs. MR, respectively. CR for EUS between TR vs. FD and TR vs MR is 90.5% and 98.4% respectively. Some of the factors for the discordance were additional material in cellblock, and ancillary studies (immunohistochemistry and flow cytometry) as evident from a fairly comparable CR between TR and MR.

	Overall	EBUS	EUS
Total Number of cases	195	132	63
Discrepant cases on final diagnoses	11	5	6
Discrepant cases on microscopic review	3	2	1
Concordance rate Telecytopathology vs. Microscopic review	98.5%	98.5%	98.4%
Concordance rate, Telecytopathology vs. Final diagnosis	94.3%	96.2%	90.5%
Non diagnostic rate	7.18%	10.6%	0%

Conclusions: Telecytopathology is useful modality in rendering preliminary assessment with significantly comparable concordance rate with on site microscopic review by a pathologist. Concordance and non diagnostic rates is fairly high for lymph node lesions (<= 10% discordance) which is higher for the EBUS than EUS procedures. Probability of getting a ND sample is higher with EBUS procedures. This information can be shared with clinicians when a preliminary diagnosis is requested for further triaging clinical management.

450 Measuring the Utility of Telecytology in Rapid Onsite Evaluation: Preliminary Diagnosis and Adequacy of Liver and Pancreatic Lesional Cytology Collected by Endoscopic Ultrasound Guided Needle Aspiration

Hira Yousaf[1], Tetyana Mettler[2], Khalid Amin[1], Jimmie Stewart[1]
[1]University of Minnesota, Minneapolis, MN, [2]University of Minnesota, Chanhassen, MN

Disclosures: Hira Yousaf: None; Tetyana Mettler: None; Khalid Amin: None; Jimmie Stewart: None

Background: Rapid onsite evaluation (ROSE), using telecytopathology is an integral component of cytopathology, which aids in better clinical care. It helps in gauging the adequacy of specimens and in preliminary assessment, which is often requested by physician on ROSE, to assist in triaging the clinical management. We conducted a prospective study to validate the utilization of telecytopathology for providing preliminary assessment and adequacy workups for liver and pancreaticobilliary (PB) sites.

Design: Cytology specimens obtained for liver and PB sites were recorded from 6/2018-12/2018. A numerical system was established for grading ranging from 0-4. 0-non diagnostic (ND), 1- Negative, 2-Atypical, 3- Suspicious and 4-Positive. Using this scheme all cases were

graded numerically at the initial telecytopathology review (TR) followed by scoring at microscopic review (MR) of the same slide by same pathologist and finally a third score was attributed on final diagnosis (FD). Concordance rates (CR) between TR, MR and FD were calculated, using major discrepancy (MD), as it depicts significant change in diagnosis, with possible implications on patient management. MD was defined as, an upgrade or downgrade of the diagnosis by two categories after revaluation at the MR or FD.

Results: There were total 46 cases, 13 from liver and 33 from PB sites. Liver had 0 cases with MD and PB had 7cases with MD. Comparison of TR and MR generated only 1 case for PB site with MD. All 7 cases (100%) were under call on initial review. Overall, CR between TR and MR is 98% and TR and FD is 85%. CR for TR versus MR was 100% for liver and 97% for PB. CR between TR and FD for liver was 100% and for PB is 79%. Non-diagnostic (ND) rate in our cohort was 0% with 0 ND specimens. Some of the factors for difference in CR for PB sites are additional material in cell block, utilization of ancillary testing and diagnostic challenges for the pancreatic lesions, especially mucinous neoplasms, as evident from comparable CR's between TR and MR.

	Overall	Liver	Pancreaticobilliary
Number of cases	46	13	33
Number of discrepant cases on microscopic review	1	0	1
Number of discrepant cases on Final Diagnosis	7	0	7
Concordance rate of Telecytopathology and Microscopic Review	98%	100%	97%
Concordance rate of Telecytopathology and Final Diagnosis	85%	100%	79%

Conclusions: Telecytoplatholgy is effective tool for ROSE and is significantly comparable to on site microscopic review by a pathologist. ND rate for liver and PB specimens is negligible. Preliminary and FD are akin for liver sites. For PB, TR is fairly comparable to FD (79% concordant), but there is 20% possibility of upgrade in diagnostic category on FD. This information can be communicated with the clinician when a preliminary assessment is requested for further triaging patient management.

451 Measuring the Utility of Telecytology in Rapid Onsite Evaluation: Preliminary Diagnosis and Adequacy of Cytology Specimens Collected by EUS and EBUS Guided Needle Aspiration

Hira Yousaf[1], Andrew Stone[1], Abdul Hanan[1], Tetyana Mettler[2], Khalid Amin[1], Jimmie Stewart[1]
[1]University of Minnesota, Minneapolis, MN, [2]University of Minnesota, Chanhassen, MN

Disclosures: Hira Yousaf: None; Andrew Stone: None; Abdul Hanan: None; Tetyana Mettler: None; Khalid Amin: None; Jimmie Stewart: None

Background: The remote analysis of cytology specimens using telecytopathology, is innate to cytopathology practice at academic and tertiary care centers. It offers the advantages of rapid assessment of specimen adequacy and reduced time to a preliminary diagnosis that may guide intra-procedural management while being less disruptive to the pathologist's workflow, potentially allowing for analysis of a larger number of cases and overall greater efficiency. To validate the performance of telecytopathology for providing adequacy assessments and preliminary diagnoses of EBUS and EUS specimens, we performed a prospective study at our institute.

Design: All the specimens obtained from the EUS and EBUS procedures between 6/2018-12/2018 were recorded and analyzed. A numerical scoring scheme was utilized for analysis, ranging from 0-4. 0-non diagnostic (ND), 1- Negative, 2-Atypical, 3- Suspicious and 4-Positive. All the cases were graded utilizing this scheme at the initial telecytopathology review (TR), followed by the microscopic review (MR) of the same slide by the same pathologist and lastly on the final diagnosis (FD). Concordance rates (CR) between TR, MR and FD were calculated using major discrepancy (MD), since MD is indicative of notable difference in the diagnostic category, potentially influencing clinical management. MD was defined as an upgrade or downgrade of the diagnosis by two categories after revaluation at the MR or FD.

Results: EBUS had a total of 154 cases. 6 cases had a MD between TR and FD and out of those 2 cases were discrepant between TR and MR. CR between TR and MR is 98.7% and TR and FD is 96.1%. EUS has a total of 135 cases. 13 cases were with MD between TR and FD and out of those 2 cases had MD between TR and MR. CR between TR and MR is 98.5% and TR and FD is 90.4% for EUS specimens. There are 18 non-diagnostic cases for EBUS and 0 for EUS specimens with a ND rate of 11.7 % for EBUS and 0% for EUS. Please see the table for detailed data/results.

	EBUS		EUS			
	Lungs	Lymph Nodes	Lymph Nodes	Liver	Pancreatic billiary	Misc.
Total Number of cases	22	132	63	13	33	26
Discrepant cases Telecytopathology vs. Microscopic review	0	2	1	0	1	0
Discrepant cases telecytopathology vs. final diagnosis	1	5	6	0	7	0
Concordance rate of telecytopathology and Microscopic review	100%	98.5%	98.4%	100%	97%	100%
Concordance rate of telecytopathology and Final diagnosis	95.45%	96.2%	90.5%	100%	79%	100%

Conclusions: TR is fairly comparable to MR in initial assessment and rendering a preliminary diagnosis, promoting use for adequacy workups and preliminary assessment. ND rates are low at our institute with a probability of having a ND specimen higher for EBUS procedures than for EUS procedures. CR between TR and FD is higher for EBUS procedures than EUS procedures. These results are important for clinicians to know prior to requesting preliminary results for patient communication or therapeutic action.

452 Cellular Mechanical Analysis as a Biomarker for Urothelial Carcinoma

Weibo Yu[1], Neshat Nilforoushan[2], Michael LeClaire[1], Shivani Sharma[3], Jianyu Rao[1]
[1]University of California Los Angeles, Los Angeles, CA, [2]Irvine, CA, [3]California NanoSystems Institute, University of California, Los Angeles, Los Angeles, CA

Disclosures: Weibo Yu: None; Neshat Nilforoushan: None; Michael LeClaire: None; Shivani Sharma: None; Jianyu Rao: None

Background: Urothelial cancer (UC) is the most common malignancy in the urinary tract. Current urine cytology diagnosis of UC can be challenging. Furthermore, there is no reliable biomarker for the distinction of non-invasive from invasive UC. Cellular mechanical property has been recognized as an important biomarker for cancer progression, especially from non-invasive to invasive/metastatic cancers. In this project, we first studied the association of cellular mechanical property changes with the progression of UC using an established multi-step UC model. We then tested whether cellular mechanical analysis can be performed on patient's urine cytology specimens.

Design: The *in-vitro* UC model includes non-tumorigenic/untransformed BC cells, tumorigenic/untransformed PC cells and transformed MCT11 cells that can further progress to high-grade urothelial carcinoma by carcinogen 4-ABP exposure. We profiled gene expression of model cells and characterized cell mechanical property using atomic force microscope (AFM)-based cell modular measurement. We then performed mechanical property analysis of urinary cells from six fresh urine samples, including three samples with positive cytology diagnosis and three samples with negative cytology.

Results: From non-tumorigenic urothelial BC cell, tumorigenic urothelial PC cell to MCT11 cell, cell stiffness progressively decreased (BC vs PC, cell modulus: 19.5±2.6 KPa vs 11.2±4.5 KPa, $P<0.05$, t test; BC vs MCT11, cell modulus: 19.5±2.6 KPa vs 6.7±2.2 KPa, $P<0.05$, t test). Malignant PC cell and MCT11 cell demonstrated activation in MAPK1 signaling (IPA analysis, PC vs BC: Activation z-score: 7.36, $P < 0.0001$; MCT11 vs BC: Activation z-score: 3.669, $P < 0.0001$) and other cancer related pathways, conveying the parallel changes in decreased cell stiffness and increased malignant potential.

We established standard protocols to measure cell mechanical property on urine cytology specimen with a simple cyto-centrifugation procedure. Un-fixed urine samples as old as two days after collection can still be analyzed. Figure 1B showed the results of AFM analysis of six urine samples. Cells from positive cytology samples had substantially less stiffness than samples with negative cytology.

Figure 1 - 452

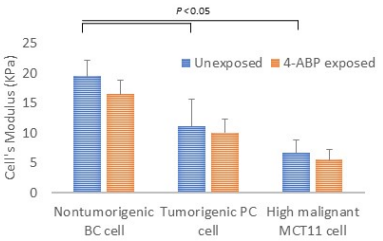


Figure 1A **Characterizing mechanical property of urothelial cells in different carcinogenesis stages**

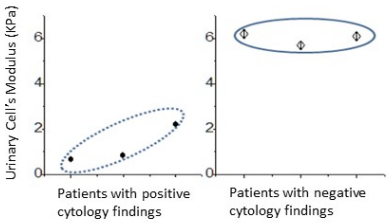


Figure 1B **Preliminary AFM-based cell modulus analysis data from urine cytology specimens**

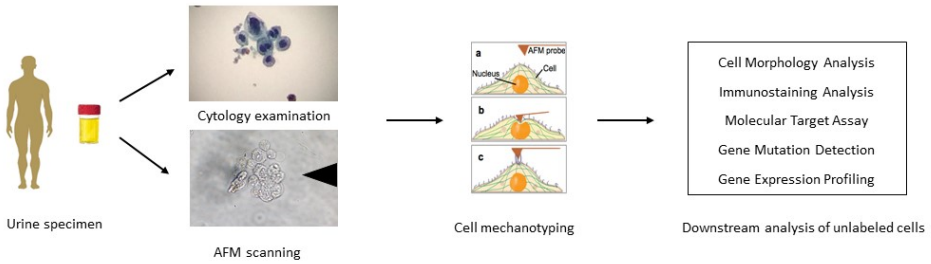


Figure 1C **Workflow of AFM-based cell modulus analysis for urine cytology specimen**

Conclusions: Cell mechanical property can be analyzed by AFM. Further studies can be performed to determine whether cell mechanical analysis can be used as a biomarker for not only detecting UC but also determining the presence or absence of tumor invasion.

453 The Role of US Guided Fine Needle Aspiration of Thyroid Bed Lesions and Clinical Predictors of Recurrent Papillary Thyroid Carcinoma

Lisi Yuan[1], Deborah Chute[2]
[1]Cleveland Clinic, Cleveland, OH, [2]Cleveland, OH

Disclosures: Lisi Yuan: None; Deborah Chute: None

Background: Fine needle aspiration (FNA) of thyroid bed lesions after thyroidectomy are challenging to evaluate. We determined the sensitivity, specificity, positive and negative predicative value of thyroid bed FNA (TB-FNA) for detecting local recurrence of thyroid carcinoma.

Design: A retrospective search for TB- FNAs over 17 years was performed. Requirements included: prior thyroidectomy (lobectomy or total thyroidectomy), and subsequent ipsilateral FNA from the “thyroid” or “thyroid bed”. Clinical and pathologic data was retrieved from the medical records. Aggressive variants of papillary thyroid carcinoma (PTC) included tall cell variant, hobnail cell variant, and poorly differentiated. Adverse features at the time of thyroidectomy included extrathyroidal extension, positive margin, or lymph node metastasis. Patients were ultimately classified as “malignant” or “benign” based on the worst pathology identified. If no histologic follow-up was available, stable clinical monitoring of at least 6 months with a clinical impression of a benign process classified patients as “benign”. P values were calculated using t-test and Fisher Exact test.

Results: 41 cases (3:1 M:F) were included (mean age: 55 years). Median interval between surgery and TB-FNA was 39 months. Prior thyroidectomy pathology included: 35 PTC (3 incidental microcarcinomas), 2 follicular carcinomas (FC), 1 medullary carcinoma (MC), and 3 benign. TB-FNA was adequate in 37 (90%) cases, and interpreted as: positive for malignancy (21; 51%), suspicious (2; 4.8%), SFN (2; 4.8%), AUS (2; 4.8%), and benign (10; 24%). See table for cyto-histologic correlation; briefly, 26 patients had histologic follow-up, and 23 (88%) showed recurrent malignancy (21 PTC, 1 FC, 1 MC). The cytology sensitivity, specificity, positive predictive value, negative predictive value, and accuracy were 91.3%, 100%, 100%, 81.8%, and 93.8%, respectively, for identification of recurrent malignancy. Comparison of data between patients classified as “malignant for PTC” and “benign” identified clinical factors that are helpful to cytopathologists when reviewing a TB-FNA (See table).

Cytologic-Histologic Correlation of TB-FNA with Subsequent Resection (n=26)				
Cytology Result		Histologic Follow-up after TB-FNA		
		Benign	Malignant (type)	
Non-diagnostic		1	1 (1 PTC)	
Benign		2	2 (2 PTC)	
AUS		0	0	
SFN		0	1 (1 FC)	
Suspicious		0	1 (1 PTC)	
Positive		0	18 (17 PTC, 1 MTC)	
Comparison of Clinicopathologic Features in Patients with Prior PTC diagnosis (n=33) and Final Classification of Benign or Malignant after TB-FNA				
		Benign (n=9)	Malignant (n=24)	P value
Age (mean)		44 y	59 y	0.03
Female		9 (100%)	15 (62.5%)	0.05
Original Pathology	PTC size (mean)	1.1 cm	2.3 cm	0.03
	Aggressive variant	1 (11%)	6 (24%)	0.64
	Adverse features	2 (22%)	20 (95%)	<0.01
Interval to TB-FNA (mean)		57 months	32 months	NA
RAI treatment (yes)		3 (33%)	18 (75%)	0.04
Labs at time of TB-FNA	TSH high (>5.5)	1 (11%)	4 (16.7%)	1.00
	T4 low (<0.7)	0 (0%)	0 (0%)	1.00
	TG level (mean)	11.3 (range 0.2-65)	296 (range 0.2-6,000)	NA
	TG >2	1 (11%)	17 (71%)	<0.01
Imaging Features at time of TB-FNA	Increased vascularity	1 (11%)	2 (8.3%)	1.00
	Microcalcifications	0 (0%)	3 (12.5%)	0.54
	Size ≥ 2cm	1 (11%)	10 (41.7%)	0.21

AUS: atypia of undetermined significance; PTC: papillary thyroid carcinoma; RAI: radioactive iodine; SFN: suspected follicular neoplasm; T4: free thyroxine; TB-FNA: thyroid bed fine needle aspiration; TG: thyroglobulin; TSH: thyroid-stimulating hormone

Conclusions: The majority of TB-FNA cases ultimately were diagnosed with malignancy on follow-up, although there may be sampling bias as not all clinically benign cases had surgical follow-up. The strongest clinical predictors of recurrent PTC at the time of TB-FNA were prior adverse features in the original PTC and high thyroglobulin level.

454 Hürthle Cell-Rich Thyroid Cytology: A Risk-Factor Model Highly Predictive of Neoplasia

Lisi Yuan[1], Tarik Elsheikh[1], Christian Nasr[1]
[1]Cleveland Clinic, Cleveland, OH

Disclosures: Lisi Yuan: None; Christian Nasr: None

Background: In the absence of definitive malignant features, it is difficult to distinguish non-neoplastic (NN) from neoplastic Hürthle cell-rich (HCR) thyroid aspirates; yet this distinction is clinically important. Prior studies have reported various cytologic or ultrasound (US) features associated with neoplasia and malignancy, but had contradicting findings. No study has previously correlated newly introduced ATA and TI-RADS ultrasound scoring systems with cytologic features. Our aim was to identify risk factors predictive of neoplasia by correlating cytologic, clinical and US scoring systems, individually and combined, with surgical outcome.

Design: Files were searched for all HCR FNAs diagnosed from 2010-2014 with surgical follow-up and US imaging. 71 cases were identified, including 36 NN, 21 adenomas, and 14 carcinomas. US was interpreted by an endocrinologist, blinded to surgical outcome, and data recorded utilizing ATA and TI-RADS systems. Clinical parameters including age, sex, and nodule size were documented. Sixteen previously published cytologic features were evaluated blinded to cyto-histologic diagnoses, including cellularity, admixed follicular cells, microfollicles, flat sheets vs. 3D, isolated cells, uniform population or anisonucleosis (non-uniform), small/large cell dysplasia, colloid, lymphocytes, transgressing blood vessels, intracytoplasmic lumina, PTC features, and cystic change; each cytologic feature was semiquantitatively scored. All cytologic, US, and clinical data were evaluated by univariate, multivariate and stepwise logistic regression analysis; and statistical significance achieved at P-value < 0.05.

Results: On univariate analysis, significant predictors of neoplasia were high cellularity, isolated cells, absent colloid, anisonucleosis, larger nodule size, and higher ATA risk. Multivariable model selection for neoplasm identified 4 risk factors (high cellularity, anisonucleosis, absent colloid, and size >2.9 cm), when present in combination, were able to predict neoplasm 84.2% of the time compared against

randomly selected non-neoplastic cases. No patients without any of these risk factors had neoplasm, while 91% with all 4 risk factors had neoplasm. When compared against ATA and TI-RADS scoring systems, this model predicted neoplasm significantly better.

Table. Multivariable Model Predictive of Neoplasm & Comparison to Ultrasound Imaging Scoring Systems

4-risk factor model		
Risk Factor	**OR (95% CI)**	**p-value**
Colloid absent	6.84 (1.71, 27.32)	***0.006***
Size >2.9 cm.	5.45 (1.58, 18.77)	***0.007***
Anisonucleosis	4.81 (1.38, 16.76)	***0.014***
Cellularity high	3.95 (1.08, 14.47)	***0.038***
Comparison of Model with Other Scoring Systems		
Scoring system	**AUC (95% CI)**	**P-value (vs 4-risk factor model)**
4-risk factor model	0.842 (0.750, 0.935)	**N/A**
ATA	0.679 (0.558, 0.800)	***0.040***
TI-RADS	0.598 (0.471, 0.725)	***0.005***

ATA: American Thyroid Association ultrasound classification system; AUC: area under curve; CI: confidence interval; N/A: not applicable; OR: odds ratio; TI-RADS: Thyroid Imaging Reporting And Data Systems.

Figure 1 - 454

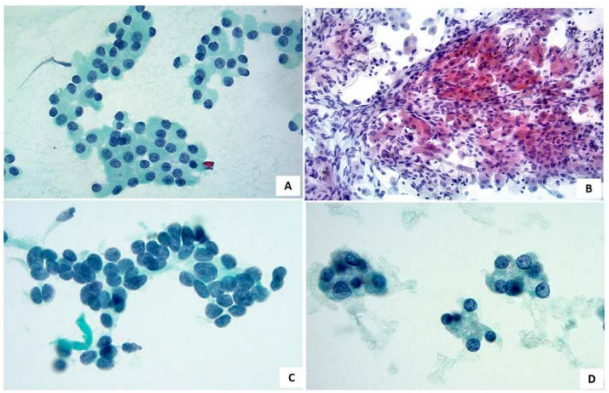


Figure 1. Some of the cytologic features that were not statistically significant in multivariate analysis in distinguishing neoplasm from non-neoplastic conditions. A: Uniform cell population. B: Transgressing blood vessels. C: Small cell dysplasia. D: Prominent microfollicle formation.

Figure 2 - 454

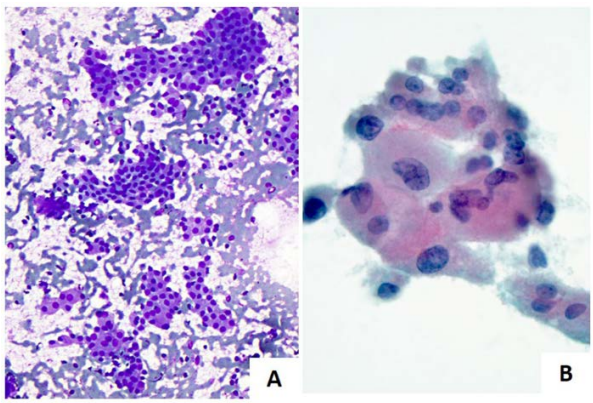


Figure 2. Cytologic features found to be statistically significant in predicting neoplasia in Hürthle cell-predominant aspirates. A: High cellularity and absent colloid. B: Anisonucleosis (Non-uniform cell population).

Conclusions: Our study identified 4 risk factors, when present in combination, predicted neoplasm in HCR cytology better than US imaging scoring systems or other clinical factors.

455 Detection of Cervical Squamous Dysplasia on Cell Blocks Prepared from Pap Test Samples - A Combined Study Based on Morphology, HPV Detection by RNA In Situ Hybridization, and Immunohistochemical Stains for P16 and Ki-67

Michele Zelonis[1], Jianhui Shi[1], Haiyan Liu[1], Robert Monroe[2], Fan Lin[1]
[1]Geisinger Medical Center, Danville, PA, [2]Advanced Cell Diagnostics, San Mateo, CA

Disclosures: Michele Zelonis: None; Jianhui Shi: None; Haiyan Liu: None; Robert Monroe: *Employee*, Bio-Techne; Fan Lin: None

Background: The Pap test is a screening procedure for cervical cancer. Colposcopic examination/biopsy is recommended for all patients, regardless of age, with a diagnosis of high-grade squamous lesion (H-cells) or atypical squamous cells, cannot exclude a high-grade lesion (ASC-H), and for patients over 24 years of age with a diagnosis of low-grade squamous lesion (L-cells). Recent studies demonstrate that RNA in situ hybridization (RNA ISH) technology is a highly sensitive and specific method for the detection of both high-risk (HR) and low-risk (LR) types of HPV on cervical biopsies. In this study, we propose a one-step approach to a conclusive diagnosis from a Pap test sample by using a combined cell block (CB) preparation, immunostains for p16 and Ki-67, and detection of HPV by RNA ISH

Design: Forty-six CBs were prepared from material remaining from Pap tests after the cases were signed out. These included H-cells (N=10), L-cells (N=11), ASC-H (N=9), negative/benign (N=12), atypical glandular cells of undetermined significance (AGUS) (N=2), and

endometrial (EM) cells (N=2). No ASC-US cases were included because the remaining Pap test materials were submitted for molecular HPV testing. Immunostains for p16 and Ki-67 and RNA ISH for 18 HR HPV types and 6 LR HPV types were performed on each case. The diagnosis was rendered for each case based on the morphology of the CB in conjunction with the staining results (p16 and Ki-67), and HPV RNA ISH.

Results: Forty-two of the 46 cases demonstrated sufficient cellularity for evaluation. One case from each group (H-cells, L-cells, ASC-H, and EM-cells) was excluded due to inadequate cellularity. All 10 L-cells cases were diagnosed as low-grade dysplasia. The AGUS and EM-cells case were negative for dysplasia. The results for H-cells and ASC-H groups with available cervical biopsies are summarized in Table 1. The increased Ki-67 index was seen in 50% of these cases. A representative case of high grade squamous dysplasia is shown in Figure 1.

Table 1. Summary of study results on H-cells and ASC-H groups

Pap Test	HPV RNA ISH	p16	Cell Block	Cervical Biopsy
H	+	+	HG	NA
H	+	+	HG	NA
H	+	+	HG	ND
H	+	+	AEC	CIN 1
H	+	-	HG	NA
H	+	+	HG	CIN 3
H	+	+	HG	CIN 3
H	+	+	HG	CIN 3
At least L-cells	+	-	LG	CIN 1
ASC-H	+	+	HG	CIN 3
ASC-H	+	+	HG	CIN 2
ASC-H	+	-	HG	CIN 3
ASC-H	Negative	-	ND	ND
ASC-H	+	-	LG	CIN 3
ASC-H	+	+	HG	CIN 3
ASC-H	+	+	HG	NA
ASC-H	+	+	HG	NA

Note: H – H-cells; NA – not available; HPV RNA ISH – for high risk types HPV; ND – no dysplasia; HG – high-grade dysplasia; AEC – atypical endocervical cells; LG – low-grade dysplasia; CIN – cervical intraepithelial neoplasia.

Figure 1 - 455

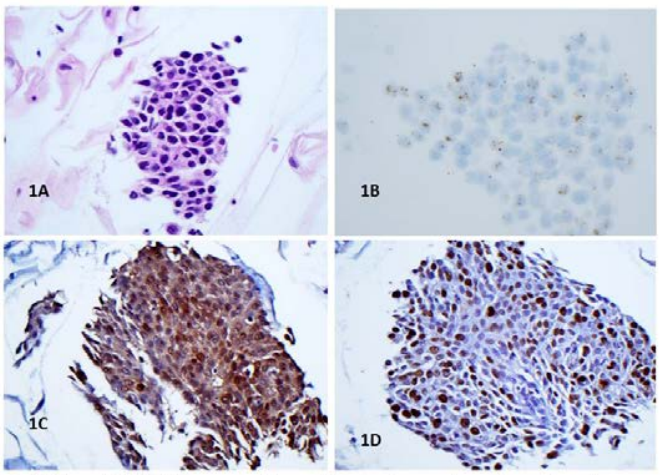


Figure 1 (1A through 1D) showing a representative case of H-cells on Pap test, diagnosed as a high grade squamous lesion on cell block and confirmed as CIN III on the follow-up cervical biopsy. Note that dysplastic squamous epithelium on the cell block preparation (1A), positive for high risk types of HPV by RNA ISH (1B), diffusely positive for p16 (1C), and the increased Ki-67 index (1D).

Conclusions: Our studies demonstrated that using cell blocks prepared from materials remaining from Pap test samples, in conjunction with detection of HPV by RNA ISH and immunostains for p16 and Ki-67, can provide a definitive diagnosis as a cervical biopsy. If this approach can be validated in a large multi-institutional study, a diagnosis of squamous lesion from a Pap test can be viewed as the final diagnosis. Therefore, a colposcopy procedure with a cervical biopsy to confirm the Pap diagnosis can potentially be eliminated.

456 Lymph Node Metastasis False Negative Percentages as a Function of Node Size, Percent Replacement of Lymph Node and Tumor Deposit Size

Tao Zhang[1], Magda Esebua[2], Lester Layfield[3]
[1]University of Missouri School of Medicine, Columbia, MO, [2]University of Missouri, Columbia, MO, [3]Columbia, MO

Disclosures: Tao Zhang: None; Magda Esebua: None; Lester Layfield: None

Background: Fine needle aspiration (FNA) is commonly used to investigate lymphadenopathy of suspected metastatic origin. While diagnostic accuracy of FNA for lymph node disease is well described, the relationship between node size, percent tumor replacement, and size of metastatic deposit with diagnostic accuracy is less well documented.

Design: All axillary lymph nodes undergoing ultrasound-guided FNA for suspected breast metastases were correlated with subsequent surgical excision specimens. FNAs were judged as positive or negative for malignancy and the percent of false negative FNAs was correlated with node size, percent tumor replacement and size of metastatic deposit

Results: One hundred and seventy-three axillary node FNAs were performed and forty-five were associated with surgically confirmed nodal metastases. False negative percentages were calculated for nodes greater than 2cm (20%, 1/5), nodes 1-2 cm (22%, 5/23), nodes 0.5 to 0.99 cm (27%, 4/15) and for nodes less than 0.5 cm (100%, 2/2). Percentage tumor replacement was correlated with false negative percentage: greater than 75% replacement (24%, 4/17) 51 to 74% replacement (22%, 2/9), 25 to 50% replacement (31%, 4/13) and less than 25% replacement (33%, 2/6). Metastases size was also correlated with percent false negative FNAs: deposits greater than 2cm (25%, 1/4) 1 to 2cm (0%, 0/12), 0.5 to 0.99cm (30%, 3/9) and less than 0.5cm (40%, 8/20).

Conclusions: Percentage of false negative FNAs associate with investigation of metastatic disease correlates with node size, size of metastatic deposit and percentage of node replaced by tumor. Lymph nodes smaller than 5mm, deposit diameter less than 5mm and percentage replacement of less than 25% have the highest percentage of false negative results.

457 Developing Machine Learning Algorithm for Urine Cytological Diagnosis of Urothelial Carcinoma

Zhihui Zhang[1], Jiwei Liu[2], Fang Fengqi[3], Jianyu Rao[4]
[1]National Cancer Center/National Clinical Research Center for Cancer/Cancer Hospital, Chinese Academy of Medical Sciences and Peking Union Medical College, Beijing, China, [2]1st Affiliated Hospital of Dalian Medical University, Dalian, Liaoning, China, [3]The First Hospital Dalan Medical University, Dalian, China, [4]University of California Los Angeles, Los Angeles, CA

Disclosures: Jiwei Liu: None; Fang Fengqi: None; Jianyu Rao: None; Jianyu Rao: *Speaker*, Zhiwei

Background: Urine cytology for the diagnosis of urothelial carcinoma (UC) can be challenging, especially for low grade UC. An AI-based machine learning tool, MorphoGo (ALAB, Boston, MA, USA) armed with automatic acquisition/scanning, optical focusing, and automatic classification with Convolutional Neural Network (CNN) has been developed. The system has been showing the utility in bone marrow smear analysis for hematopoietic disorders. The goal of the project is to determine whether an algorithm can be developed for urine cytological analysis of UC.

Design: A total of 37 abnormal urine cytology cases with atypical urothelial cells (AUC) and above diagnosis were obtained from CHCAMS and used for training, among which 27 were also used for validation. Each cell acquired was classified into one of the following groups: Degenerated Cells, Blood and Inflammatory Cells, Benign Epithelial Cells, Atypical Cells, Suspicious Cells, and Others. During the initial training phase, cells were assigned into the above category by one pathologist (JY Rao). Additional 12 cases were collected from FAHDMU for testing without knowing patient's history.

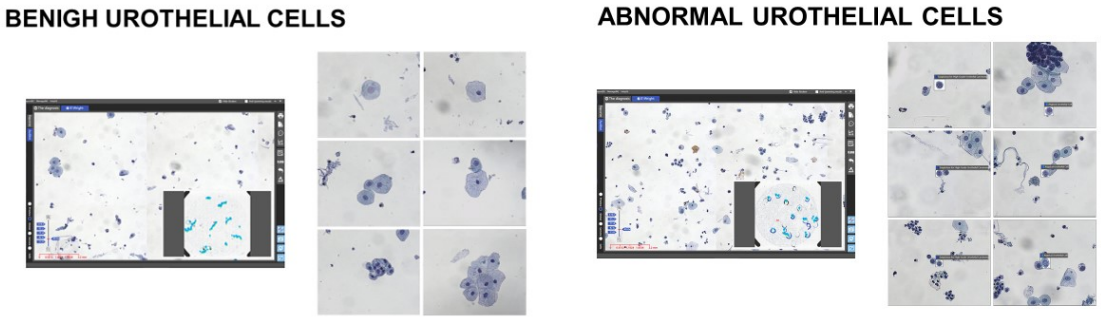
Results: A total of 1978 cells were acquired during the initial training phase and 436 cells were acquired and analyzed for validation by MorphoGo. Fig 1 showed examples of benign urothelial cells versus abnormal (atypical and suspicious) urothelial cells identified by MorphoGo based on the algorithm. Of the 12 unknown cases preliminary tested (Table 1), 6 had HGUC, 1 LGUC, 1 prostate adenocarcinoma, 1 renal cell carcinoma, and 4 with non-neoplastic conditions. The original cytology was positive for tumor cells in two HGUC samples, and MorphoGo was abnormal (AUC, Suspicious and Positive) for five samples from patients with UC malignancy, including one with LGUC. Notably, MorphoGo also identified abnormal cells in the case with prostate adenocarcinoma.

Table 1. Results of Initial Cytology Diagnosis, MorphoGo, and Histological Diagnosis

Case No.	Cytology diagnosis from original institution	MorphoGo	Histological Diagnosis
1	Positive for Tumor Cells	Susp./Pos.*	HGUC**
2	Unknown	Other abnormal cells	Prostatic cancer CA
3	No tumor cells	Susp.	LGUC
4	No tumor cells	-	HGUC
5	A few heteromorphic cells	Susp./Pos.	HGUC
6	No tumor cells	-	Renal calculus
7	No tumor cells	-	HGUC
8	Individual abnormal cells	AUC	Right renal cyst. Bilateral renal calculi.
9	Positive for Tumor Cells	Susp./Pos.	HGUC
10	No tumor cells	AUC	HGUC
11	Unknown	-	Right renal carcinoma
12	Rare abnormal cells	-	Prostatic hyperplasia with inflammation

*Positive with >= 10 suspicious cells **HGUC, High grade urothelial carcinoma; LGUC, Log grade urothelial carcinoma; AUC, Atypical urothelial cells

Figure 1 - 457



Conclusions: An algorithmic approach developed by MorphoGo has the potential to be used for urinary cytological diagnosis of UC. Further refinement and validation with larger volume of urine samples is warranted.

458 Effect of Preanalytic Variables on Interpretation of MTAP Immunohistochemistry on Cell Blocks

Xiaoqin Zhu[1], Marina Vivero[2]
[1]Brigham and Women's Hospital, Shrewsbury, MA, [2]Brigham and Women's Hospital, Boston, MA

Disclosures: Xiaoqin Zhu: None; Marina Vivero: None

Background: Homozygous deletion of the 9p21 locus is 100% specific for the distinction between malignant pleural mesothelioma (MPM) and reactive mesothelial hyperplasia (RMH). Recent studies have shown that loss of methylthioadenosine phosphorylase (MTAP) protein expression is a sensitive and specific surrogate for 9p21 deletion, and demonstrates 45% and 42% diagnostic sensitivity for mesothelioma in surgical and cytology specimens, respectively. Our aim is to study the impact of specimen age and cellularity on interpretation of MTAP immunohistochemistry (IHC) in cell block specimens, and correlate to MTAP expression in matched surgical specimens.

Design: Included were pleural effusions with a diagnosis of MPM or RMH and corresponding surgical specimens collected over a 14-year period. Patients with thoracic carcinomas were excluded. Archival paraffin-embedded materials from all cytology cases and, when available, surgical cases were stained with anti-MTAP antibody (Santa Cruz, 42-T, 1:75). Semiquantitative assessment of cellularity (<50, 50-100, 101-200, and >200 cells) and percentage of cells demonstrating cytoplasmic loss of MTAP protein expression was performed on cytology cell blocks.

Results: 59 cell blocks from 40 men and 19 women with a median age of 69 (Q1-Q3=62-74), comprising 45 mesotheliomas and 14 benign effusions were included. 25 (56%) of mesothelioma effusions and 1 (7%) benign effusion demonstrated loss of MTAP expression. The

benign case demonstrating loss was from 2014, with <50 cells and a weak internal control, and was associated with no development of pleural disease over 5 years of follow-up. Among 23 cases with matching surgical specimens, concordance was 96%. The single discordant cell block contained 50-100 mesothelial cells with faint cytoplasmic staining and corresponded to a biphasic mesothelioma with MTAP loss. The diagnostic sensitivity and specificity of MTAP loss for mesothelioma in cell blocks is 56% and 93%, respectively. Loss of MTAP expression was not affected by specimen age and was lost in 88%, 55%, and 41% of cases from 2005-2010, 2010-2014, and 2015-2019, respectively (p=0.094). Loss of MTAP staining did not significantly differ with respect to specimen cellularity (p=0.229).

Conclusions: MTAP is a useful ancillary diagnostic test for mesothelioma in effusion cytology, with a sensitivity of 56%, and can be successfully used in older and pauci-cellular cell blocks. Attention to internal controls in cell block specimens is important in pauci-cellular specimens.