Journal of the American Society of Cytopathology (2017) 6, S79–S84

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MORNING PLATFORM PRESENTATIONS

November 11, 2017, 11:00 am – 12:00 pm

MORNING PLATFORM PRESENTATIONS

PL01

The Use of FTA Cards as a Cheap and Simple Tool to Store DNA from Pancreatic Endoscopic Ultrasound Guided Fine Needle Aspirates

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Introduction: In the current era of targeted therapy and rising of novel biomarkers in pancreatic carcinomas, simple and efficient methods of DNA storage and extraction are urgently needed. The objective of this study was to determine the utility of FTA cards in storing DNA from endoscopic ultrasound (EUS) guided fine needle aspirates (FNA).
Materials and Methods: EUS-guided FNA of solid pancreatic lesions was performed using 22 gauge-needles. After all the diagnostic workup, the remaining material in the needle was rinsed in saline and subsequently centrifuged, with three drops from the pellet applied on FTA cards. DNA was extracted from the cards using Qiagen DNAeasy Micro Kit. PCR was performed using primers for the HFE gene (S65C mutation), with a resulting amplicon of 251bp. Successful reaction was determined by the visualization of bands in the agarose gel.
Results: Five patients (3 male, 2 female, median age 56 years old) were included in the study. Diagnoses included three pancreatic ductal adenocarcinomas and two mucinous cystic lesions. The average DNA concentration was 1.9ng/µl (range 1.3-2.6), with an average 260/280 purity ratio of 1.7 (range 1.51-2.16). All the cases were successfully amplified, with visualization of clear bands for all samples.
Conclusions: EUS-guided pancreatic FNA samples stored on FTA cards yielded good quality DNA, with successful amplification of all cases. These limited specimens obtained from minimally invasive procedures could be potentially used in various molecular studies, what augments the material currently available for the study of the complex molecular pathways involved in pancreatic carcinomas.

PL02

Circulating Tumor Cell Detection via a Novel FISH Assay Prior to Lung Biopsy Enables Accurate Prediction of Pulmonary Malignancy: Results of a Liquid Biopsy Study in Seventy-two Patients

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Introduction: Lung cancer is the leading cause of cancer death in the United States. Lung cancer may present as indeterminate pulmonary nodules. However, due to cost, morbidity, and high rate of negative biopsies, many patients are followed by CT scans alone. Circulating tumor cells (CTCs) may be found in the peripheral blood of non-small cell lung cancer (NSCLC) patients. Our prospective study used FISH probes, which were developed based on data derived from CGH arrays in NSCLC, to detect CTCs.
Materials and Methods: Patients with no prior history of lung cancer and an indeterminate lung nodule(s) were eligible. Blood was collected prior to biopsy. I-FISH was performed blinded on enriched peripheral blood mononuclear cells using a 3q tel, 3p22.1, cep10, 10p22.3 custom probe set. Intact cells (500) were analyzed by an automated instrument optimized to select for larger cells, and sub classified based on gains and /or losses of fluorescent signals. CTCs were defined as cells with increased copy number of $\geq$ 2 genes. A positive assay was defined as $\geq$ 2 CTCs; negative assay was CTCs <2. The biopsy was used as the gold standard.
Results: See tables 1-4.
Conclusion: This is the first diagnostic biomarker to accurately detect early lung cancer, as an adjunct to an indeterminate lung nodule, and hence has tremendous clinical utility. This CTC test has been validated in a second lab to be highly accurate and may in the future, substitute for a conventional biopsy. We noted that positive CTC tests were associated with malignancies other than lung cancer, but these had a greater false negative rate. Patients with a positive CTC test and an indeterminate lung nodule should be sent for FNA and/or biopsy. Conversely, patients with a negative CTC test can avoid a potentially harmful biopsy and be followed clinically and by imaging studies.

Total cases	72
True positive	41
True negative	19
False positive	2
False negative	10

* False negatives can occur with tumors other than NSCLC such as melanoma

Table 1 Results

True positive non-small cell lung cancer	36/39 cases
False negative lung cancers	3/39 cases -2 neuroendocrine carcinomas -1 squamous cell carcinoma

Table 2 Lung cancers

Accuracy for diagnosing non-small cell lung carcinoma	97%
Accuracy for all lung cancer	92%

Table 3 Accuracy

2213-2945/$36 © 2017 Published by Elsevier Inc. on behalf of American Society of Cytopathology.
http://dx.doi.org/10.1016/j.jasc.2017.06.194

Sensitivity	80.4%
Specificity	90.5%
Positive predictive value	95.3%
Negative predictive value	65.5%

Table 4 All malignancies

PL03

The Role of hrHPV Genotyping in Risk Assessment among Cytology Diagnosis Categories: Analyzing 4732 Women with Cytology-HPV Cotesting and Follow-up Biopsy

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Introduction: HPV detection and genotyping have been used in clinical risk assessment. The purpose of this study was to analyze the performance of HPV genotyping in risk evaluation among cytology diagnostic categories.

Materials and Methods: Between January 1, 2015 and December 31, 2016, 4732 Pap tests samples (ThinPrep 2648, SurePath 2084) with cervical biopsies were analyzed along with HPV results. The cytology diagnoses include NILM (n=1015), ASC-US (n=1668), AGC (n=126), ASC-H (n=199), LSIL (n=1514) and HSIL+ (HSIL or worse, n=174). The samples were tested on Cobas (n=4105) or Aptima (n=627) platforms. The biopsies were classified into three groups: benign (n=1905), LSIL (n=2116) and HSIL+ (n=711, including CIN2/3, AIS and carcinomas). All CIN2 lesions were confirmed by p16/Ki-67 immunostains. Endometrial lesions were excluded from the study.

Results: Detection of HPV 16/18/or45 was associated with significantly higher rates of HSIL+ biopsy lesions across all cytology diagnostic categories ($p<0.001$, respectively). However, specificity HPV genotyping for HSIL+ biopsy lesions was low in ASC-H and HSIL categories (77.8% and 76.9%, respectively) compare to that in ASC, AGC and LSIL categories (97.4%-100%). HSIL+ biopsy lesions were associated with significantly higher rates of positive Aptima testing than Cobas testing when comparing HPV16/18/or45 (45.5% vs. 24.4%, $p<0.0001$), non-16/18/or45 hrHPV (17% vs. 11.4%, $p=0.007$), or combined (25.1% vs. 15.9%, $p<0.008$).

Conclusions: hrHPV genotyping were sensitive for HSIL+ biopsy lesions in all cytology categories. However, its triaging role was greatly diminished in ASC-H and HSIL categories due to low specificity. The findings support the ASCCP recommendation of using HPV genotyping for clinical risk assessment. In addition, the performance of Aptima hrHPV genotyping in risk stratification was superior to Cobas assay due to its strong association with HSIL+ biopsy lesions. The mechanism may be related to the significant increase in E6/E7 expression following HPV DNA integration in HSIL+ lesions.

PL04

Comparison of PD-L1 Immunostaining for Pancreatic Ductal Adenocarcinoma between Paired Cytological and Surgical Specimens

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Introduction: Pancreatic ductal adenocarcinoma (PDA) has a poor prognosis, and many patients are not diagnosed until the extent of their disease precludes surgical intervention, leaving chemotherapy or palliative treatment as the only options for therapy. Success has been found in treating non-small cell lung cancers with immunotherapy for PD-1 or its ligand, PD-L1. Prediction of therapeutic response to these drugs is possible by measuring PD-L1 expression by immunohistochemistry. In many unresectable cases, cytology specimens are the only source of tissue to test the biomarkers. The aim of the study is to compare the expression of PD-L1 between paired cytology and surgical samples in PDA.

Materials and Methods: Paired formalin-fixed cell blocks and surgical specimens with confirmed diagnosis of PDA (n=28) were sectioned. Cellularity in cell blocks was counted and the cytological cases with less than 50 tumor cells were excluded for this study. Slides containing the cell block and surgical section from the same patients were immunostained with PD-L1 antibodies (DACO, California). The tumor proportion score (TPS) from both the cell block and surgical specimens were counted and analyzed.

Results: PD-L1 expression in PDA was heterogeneous (Figure 1). In surgical specimen, the PD-L1 immunostains were positive in 6 of 10 (60%) PDA including 5 cases in 1-49% category and 1 case in 50%. In cytology specimen, the PD-L1 immunostains were positive in 5 of 10 (50%) PDA. Five (50%) cytology cases matched PD-L1 immunostains with paired surgical specimen (Table 1). Inflammatory cells in surgical specimens are positive for PD-L1 in all cases.

Conclusions: PD-L1 is heterogeneously expressed by tumor cells in PDA. Only 50% of cytology cases matched with paired surgical specimens due to the heterogeneous expression of PD-L1 in PDA. Extensive and widespread sampling of the tumor may improve the detection of PD-L1 expression in cytological samples.

Figure 1: Comparison of PD-L1 immunostaining for pancreatic ductal adenocarcinoma between paired cytological and surgical specimens.

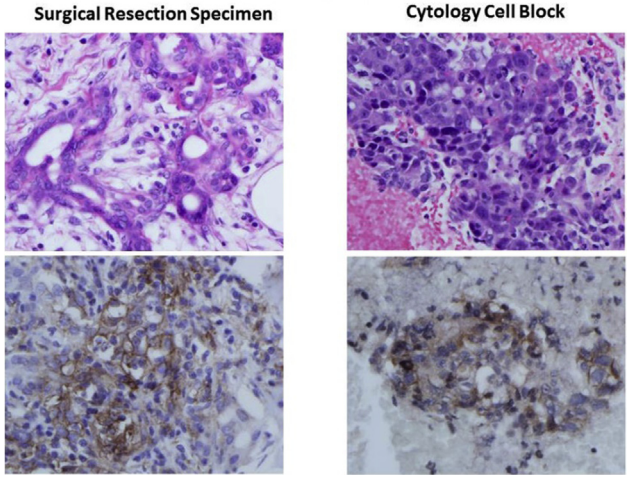

Table 1 Comparing PD-L1 expression in paired cytology and surgical samples in PDA

		Cytology specimen		
		positive	negative	total
Surgical specimen	positive	3	3	6
	negative	2	2	4
	total	5	5	10