

Non small cell lung cancer in the targeted therapy era: A cytopathologist's perspective

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There has been a dramatic change in respiratory tract cytology in past several decades due to advances in flexible bronchoscopy and fine needle aspiration biopsy. The clinicians' expectations have changed as well – it is do more with less and less.

In addition, in the past several decades primary pulmonary neoplastic disease has shifted from central squamous carcinoma to peripheral adenocarcinoma, making it more amenable to aspiration biopsy sampling.

Standard approaches to the diagnosis of lung cancer by cytology have been with conventional cytology: sputum (not good for screening), brushes, washes, lavages; and transbronchial FNA which is best for lesions below the bronchial epithelial surface. Endoscopic biopsy with ultra sound guidance (EBUS) is gaining in popularity. With these procedures biopsies the need for an additional surgical procedure is eliminated in 20% of patients. The cost is 1/3 of a mediastinoscopy.

Also in increasing use due to the shift in lung cancer to peripheral disease is transthoracic FNA which has the greatest benefit when it spares the patient an invasive procedure. It is used to document unresectable malignancy, confirm pulmonary metastases or recurrence and to diagnose lesions not treatable by surgery, such as small cell carcinoma. It also plays a role in documenting benign conditions such as hamartoma, granuloma, etc. Transthoracic FNA can avoid surgery in 30% of patients.

Transthoracic FNA should be performed with a cytotechnologist or cytopathologist present during the procedure. On site assessment is a vital part of the fellowship training experience. On site assessment will assure adequacy of the specimen and allow for special studies to be done (flow, mutational analyses, microbiology, etc).

Transbronchial FNA is very good at separating on small cell lung cancer from small cell lung cancer with a positive predictive value of 100% and negative predictive value of 70%. Conventional cytology (brushes, washes lavages) run at about a PPV of 94% for each and NPV of 30%. Interestingly, bronchial biopsy results are comparable to cytology results.

Transthoracic FNA can result in both False Positive and False Negative results; most FNs are sampling errors; reliability of a negative result is

questionable in the setting of a lung mass without a specific benign diagnosis such as granuloma, etc. Transthoracic PPV is about 98%, NPV 70%, FP rate 0.85% (*huge impact on patient*), and FN rate 8%.

In the differentiation of small cell v non small cell carcinoma, cytology is reliable in >95% of cases but this alone is no longer good enough in era of targeted therapy. Adenocarcinoma must be separated from squamous carcinoma.

Cytologic preparations can allow the distinction of adenocarcinoma subtypes, including BAC, and can recognize squamous cell carcinoma.

- Classic cytologic criteria exist to separate adenocarcinoma from squamous, but they may not often be used.
- IHC can be applied to cytology material, to cell blocks, Thin Preps and direct smears. An underreported artifact of cell blocks is a squamoid appearance that can be misleading.

Use of IHC in Pulmonary

- TTF-1 and PE10 support pulmonary primary and adenocarcinoma
- 4A4 (p63), 34bE12, support squamous carcinoma
- CK7 and CK20 useful for distinguishing primary mucinous lung tumors from GI metastasis
- Two most useful stains in limited specimens are TTF-1 and p63.

Use of cytologic material for mutational analysis

- *EGFR/KRAS* must frequent mutations studied, but ALK and others being examined
- FNA cell blocks, fluids, archival slides have all been successfully used for mutation analysis
- Touch preps may done to ascertain the adequacy of core biopsy material for studies; these samples can be treacherous
- Success rate with small biopsies and cell blocks is 93%
- Success rate with pleural, pericardial and peritoneal fluids which are routinely discarded is 76%

The success of using cytology material was highlighted in a clinical trail at MSKCC (Trial 04-103: Rebiopsy of Patients with Acquired Resistance to Epidermal Growth Factor Receptor Tyrosine Kinase Inhibitors in Non-small Cell Lung Cancer) lead by the Thoracic Oncology Service under Vincent Miller, MD. The aims of this protocol were to determine the feasibility of

rebiopsy in this setting, determine the frequency and spectrum of secondary EGFR mutations, to collect material to search for novel mechanisms of acquired resistance to EGFR TKIs.

93% (94/101) surgical pathology and FNA samples were adequate for mutation analysis; 73% (24/33) fluid cytology samples adequate for mutation analysis. Nine fluid cytology samples were insufficient or degenerated. Bone samples were challenging due to decalcification and yielded poor DNA quality. Worthy of note, FNAs performed better than cores.

A project was recently done in path at MSKCC involved assessment of EGFR mutation status in needle biopsies and cytology specimens of lung adenocarcinoma by immunohistochemistry using antibodies specific to the two major forms of mutant EGFR.

- IHC with mutation specific monoclonal Abs against exon 21 L858R and exon 19 mutant (15bp) is sensitive and specific for mutation status
- 94 lung adenocarcinomas with known *EGFR* status on resection material with corresponding needle biopsy or cytology using cell blocks or Thin preps. Distribution of cases was:
 - 47 exon 19 deletion
 - 35 exon 21 L858R mutation
 - 12 wild type

Staining with Cell Signaling Technology Abs in cytology and tissue was done using a scoring system of 0,1+ negative; 2+,3+ positive. The sensitivity was:

ex 19 (15bp only)	79%	cytology	85%	histology
ex 19 (all bp)	57%	cytology		
ex 21	66%	cytology	76%	histology

Specificity and PPV were 100%; importantly, no wild type case was reactive. These results indicated that the Abs performed with good sensitivity and high specificity, there were no FPs, and the Abs potentially eliminate need for additional biopsy for mutation studies. An unanswered question for this study and for most cytology based studies is- Would better pre-analytical methods to improve results?

In January 2006 we instituted reflex testing for *EGFR/KRAS* mutations on all lung adenocarcinomas resected at MSKCC. By reflex testing is meant: No clinical order needed; a pathology finding automatically initiates another test (example: HPV testing in cytology). Mutation data for 2007 showed 674 cases analyzed for *EGFR/ KRAS* mutations, and then reflex testing was extended to submitted as well as in house cases. Cytology cell blocks were used in 9 (1.3%) cases. By 2009, 1400 cases were studied for *EGFR/KRAS*. 70 (8%) cases were cytology (FNA, pleural, BW) material.

We are just beginning to see the potential use of cytology material in the era of targeted therapy and lung pathology has lead the way in this emerging field.