Tissue Acquisition for Biomarker-Driven Therapy of NSCLC: Case Studies & Companion Narratives to Webcast at

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Clinical Stems 1 - 3

A collaborative work with

Pfizer Oncology

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Clinical Stem 1

Nonsmoking female with new right upper lobe lung mass and three PET positive mediastinal lymph nodes[1]

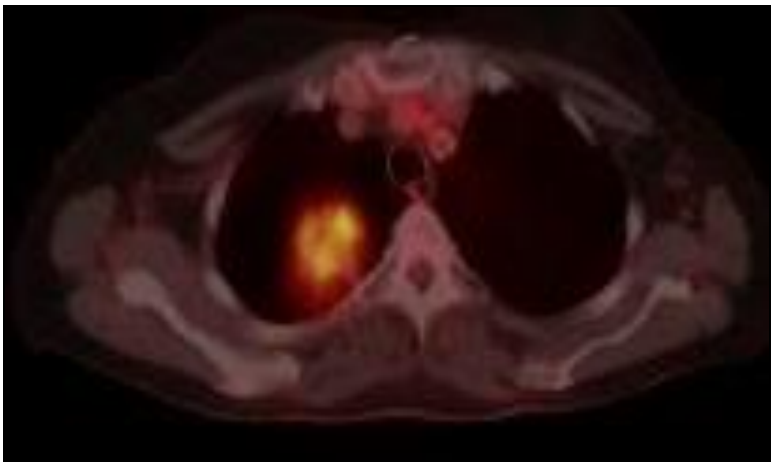
Learning objectives: the webcast participant will be able to:

1. Describe mediastinal staging and EBUS-guided nodal-sampling strategies.
2. Describe the rationale for downstream molecular analysis of small specimens.
3. Describe ways to assure specimen adequacy for diagnosis and molecular testing.
4. Describe indications for repeat biopsy in case of disease progression.
5. Describe how molecular analysis of adequate specimens helps determine prognosis and potential response or resistance to therapy.

[1] Disclaimer: This is a fictitious clinical case scenario based on a conglomerate of real patient data, modified to avoid any possibility for patient identification and to help meet educational objectives. Any resemblance to real persons, living or deceased is purely coincidental.

Case Description

A 65 year old white, nonsmoking female required preoperative medical clearance before knee surgery. Past medical history was positive for asthma since childhood. She complained of occasional dyspnea and wheezing. Physical examination was normal except for signs of left medial meniscus injury. She had minor memory loss but was independent in activities of daily living. She lived alone and had no children. Family history was unremarkable. A chest radiograph showed a 4 x 5 cm mass in the right upper lobe. Computed tomography confirmed the mass and also showed a 1.2 cm right lower paratracheal lymph node, a 1 cm left lower paratracheal node, and a 1.7 cm subcarinal node. Whole body PET CT showed increased uptake in the primary tumor and in mediastinal lymph nodes stations 4R, 7 and 4L. Brain MRI was normal.



Clinical Stem

Question 1: If this patient has primary lung cancer, which of the following most accurately predicts survival?

A. Productive cough
B. Exertional dyspnea
C. Performance status

Answer: C

Estimated survival is an important factor for decision making in all disease processes. If this patient has primary lung cancer, she would likely be clinically staged IIIB because of the contralateral mediastinal lymph node at station 4L. In such cases of advanced cancer, prognostic considerations are important because treatment goals may change from prolonging life at any cost, to palliating symptoms, preserving quality of life, and maintaining dignity.

Estimating survival based on objective data is warranted because subjective assessments of predicted survival are often incorrect and overly optimistic. Patients usually want their doctors to be realistic yet hopeful prognosticators. While exact statistics are not always shared, physicians who address prognosis can conduct meaningful and honest discussions with patients and their families.

Performance status, along with heart rate, blood pressure, temperature, respiratory rate and pain level, is an important vital sign in clinical oncology. Because performance status is the strongest prognostic indicator of survival in patients with cancer, it is frequently used as an entry criterion and adjustment factor in clinical trials. One commonly used measure of performance is the Karnofsky Performance Status score that uses a 0-100 range in ten point increments to measure functional impairment. Lower scores correlate with worsened survival for most serious illnesses.

Results from the analysis of 100 variables from several studies showed that dyspnea, dysphagia, weight loss, xerostomia, anorexia, and cognitive impairment were strongly and independently associated with cancer patient survival. These signs and symptoms were outranked, however, by the assessment of performance status.

References:

1. Lamont EB, Christakis NA. Survival estimates in advanced cancer. Available at www.UpToDate.com.
2. Hollingsworth HM. Wheezing and stridor. *Clin Chest Med* 1987;8:231–240.

Question 2: Which of the following four procedures would you perform next?

A. Mediastinoscopy
B. EBUS-guided TBNA
C. Conventional TBNA
D. Esophageal ultrasound guided FNA

Answer: B

Any of the four procedures could be performed for diagnosis, so decisions might depend on equipment availability, available expertise, or institutional bias. For lymph node station 4L, the yield is usually higher using EBUS-TBNA than using conventional TBNA. Data from a meta-analysis of EBUS-TBNA for patients with confirmed or suspected NSCLC showed that a subgroup of patients selected on the basis of CT or PET positive results had a pooled sensitivity of 94%; higher than a subgroup of patients who had not been selected according to CT or PET results.

This patient has an enlarged, PET positive lymph node. The yield of EBUS-TBNA is expected to be greater than 90%. A negative EBUS-TBNA in patients with highly suspected mediastinal nodal metastases, however, should be followed by mediastinoscopy. Mediastinoscopy allows systematic

exploration and biopsy under visual guidance of nodal stations 1, 2, 3, 4 and 7 but has associated morbidity in lesser experienced hands.

Diagnosis can probably be obtained bronchoscopically: stations 4R, 4L, and 7 can be accessed using conventional TBNA, especially using rapid on-site cytology examination, also known as ROSE. Diagnosis might also be possible using EUS-FNA to sample nodal stations 4L and 7.

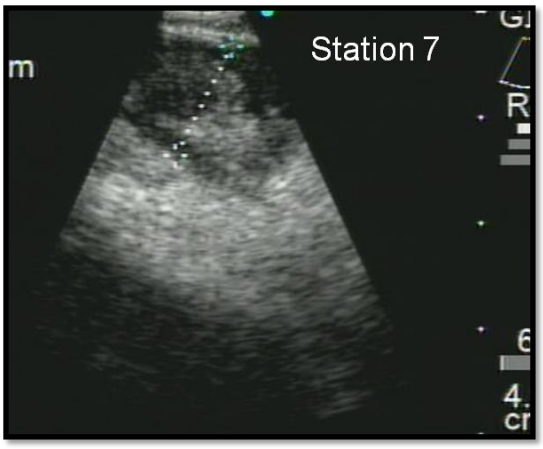
Up to 28% of patients known or suspected lung cancer and a high clinical suspicion of nodal disease might have mediastinal nodal metastases confirmed by mediastinoscopy after negative EBUS-TBNA.

References:

1. Herth FJ, Lunn W, Eberhardt R, et al. Transbronchial versus transesophageal ultrasound-guided aspiration of enlarged mediastinal lymph nodes. Am J Respir Crit Care Med. 2005 May 15;171:1164-7
2. Gu P, Zhao Y, Jiang L, et al. Endobronchial ultrasound-guided transbronchial needle aspiration for staging of lung cancer: A systematic review and meta-analysis. Eur J Cancer 2009; 45, 1389-1396
3. Defranchi SA, Edell ES, Daniels C E et al. Mediastinoscopy in Patients with Lung Cancer and Negative Endobronchial Ultrasound Guided Needle Aspiration. Ann Thorac Surg 2010; 90: 1753-8.

The Case Continues

White light bronchoscopy, EBUS-TBNA and ROSE were performed while the patient was under general anesthesia. No airway abnormalities were seen. EBUS examination of the mediastinum was normal except for enlarged nodes at levels 4L, 7, and 4R.



Question 3: Which lymph node station should be sampled first?

A. Station 4R
B. Station 4L
C. Station 7

Answer: B

Diagnostic yield from either conventional TBNA or EBUS-TBNA is highest from subcarinal station 7 nodes. While this might support initial sampling of the subcarinal node in most instances, the first station that should be sampled in this patient is station 4L because the patient has a right upper lobe mass. A positive 4L, in this case a contra-lateral mediastinal node, would confirm N3 disease. The patient might also have positive nodes at levels 7 and 4R consistent with N2 disease. Should the contralateral node be negative, than stations 7 and 4R could be sampled. If either of these N2 nodes is positive, the patient's tumor would be staged III A using the revised IASLC classification.

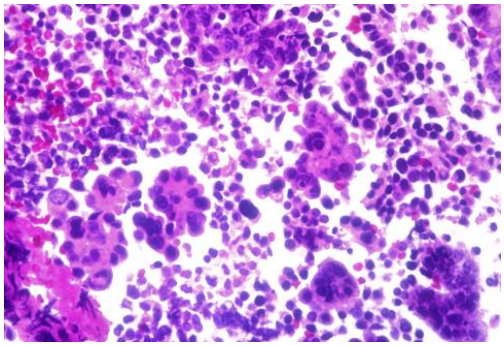
If N3 nodes are positive for malignancy on rapid on-site cytological evaluation, and if the procedure is performed for diagnosis and mediastinal staging only, the procedure could be stopped and the tumor would be staged III B.

References:

1. Rusch VW, Asamura H, Watanabe H, et al; Members of IASLC Staging Committee. The IASLC lung cancer staging project: a proposal for a new international lymph node map in the forthcoming seventh edition of the TNM classification for lung cancer. J Thorac Oncol. 2009; 4: 568–577

The Case Continues

A first aspirate was done from the level 4L node. Rapid on-site examination was positive for malignancy, favoring adenocarcinoma. Using a 22 gauge needle, the second aspirate showed core tissue. Part of the core was placed in formalin for histopathology and part was placed in stabilizing solution to be later used for DNA and RNA extraction.



Question 4: At this point you choose to:

A. End the procedure.
B. Perform at least two more aspirates from the level 4L node, and then end the procedure.
C. Perform aspirations from level 7 and level 4R nodes, and then end the procedure.

Answer: B

All choices are reasonable options. Bronchoscopists should communicate with the Pathology department in their institutions to determine a level of confidence for diagnosing lung carcinoma on rapid on-site evaluation. In general, there is a good correlation between ROSE and the final cytologic diagnosis, so ending the procedure may be appropriate. In this case, after specimen adequacy was established and the diagnosis of malignancy was made by N3 node involvement, the tumor was classified stage III B; therefore, the procedure could end after the first two aspirates. In part, this is because evidence suggests that optimal results are obtained after three aspirations per lymph node station or after two aspirations per station when at least one tissue-core specimen is obtained by the first or second aspiration.

Obtaining at least two additional aspirates from station 4L, however, is highly desirable in this patient because adequate specimen is needed for molecular analysis. Sampling station 7 and 4R is also appropriate as more tissue is needed for downstream molecular analysis. Additional samples would not be necessary for staging.

ROSE helps to confirm the presence of malignant cells and the adequate cellularity of the sample before submitting it to the molecular pathology laboratory. Some practitioners do not use ROSE because it can increase the duration and cost of the procedure. Sending the specimen directly for molecular analysis without first confirming the presence of malignancy, however, may not be cost effective because biopsy specimens may not contain sufficient carcinoma cells suitable for molecular testing.

If this patient's diagnosis is confirmed adenocarcinoma as suspected using ROSE, biomarker-directed therapy might be considered as first or second line treatment for locally advanced disease depending on biomarker analysis. Adequate specimens are therefore needed. For instance, specimens are considered adequate for EGFR analysis if they contain more than 40% malignant cells which could be obtained by four "good" fine needle aspirations or 2-3 core needle tissues.

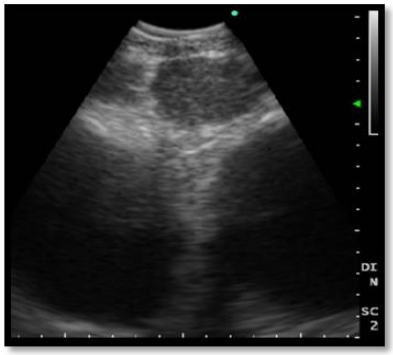
References:

1. Lee HS, Lee GK, Lee HS, et al. Real-time endobronchial ultrasound-guided transbronchial needle aspiration in mediastinal staging of non-small cell lung cancer: how many aspirations per target lymph node station? Chest. 2008;134:368-74.
2. http://labmed.ucsf.edu/uploads/369/166_24JonesMolecularPathologyOfLungCancer.pdf (accessed 8/23/11).

3. Coghlin CL, Smith LJ, Bakar S, et al. Quantitative analysis of tumor in bronchial biopsy specimens. J Thorac Oncol. 2010;5:448-52.

The Case Continues

After rapidly obtaining two more aspirates from level 4L, you chose to sample the level 7 lymph node. Using ROSE, the cytopathologists says the first aspirate at this level is also positive for malignancy and is most likely adenocarcinoma. You obtain three additional samples to send to the laboratory for molecular analysis.



Question 5: You choose to submit these samples for which of the following tests?

A. EGFR (mutation analysis)
B. KRAS (mutation analysis)
C. ALK gene rearrangement
D. All of the above

Answer: D

Results from all three tests can alter patient management. Activating EGFR mutations at exons 18-21 will predict response to Tyrosine Kinase Inhibitors, also known as TKIs. Relevant to this patient, EGFR mutation analysis is feasible in needle biopsy/aspiration paraffin-fixed specimens such as those obtained by EBUS-TBNA. EGFR mutation has been reported in 10-37% of EBUS-TBNA specimens. In one study using a PCR technique, the overall specimen insufficiency rate for EBUS was only 4%, a rate that is lower than that obtained using CT guided biopsy.

KRAS mutation occurs in approximately 15-30% of NSCLC, mostly in lung adenocarcinoma, rarely in squamous cell carcinoma and is mutually exclusive with EGFR mutations. Its presence confers resistance to treatment with TKIs. Clinically relevant mutations found in 3.5-7% of EBUS-TBNA specimens can be detected by RT-PCR or DNA sequencing. The optimal methodology for the detection of KRAS mutation for needle biopsied samples is uncertain at this time; in fact, needle specimens may be inadequate compared with resected specimens which show the mutation at higher frequencies.

Approximately 2-7% of NSCLC harbor ALK fusions and in the vast majority of cases, ALK rearrangements are non-overlapping with other oncogenic mutations such as EGFR and KRAS mutations found in NSCLC. The anaplastic lymphoma kinase (ALK) is a receptor tyrosine kinase that is aberrant in a variety of malignancies. The EML4-ALK fusion oncogene results from a small inversion within chromosome 2p. This leads to expression of a chimeric tyrosine kinase, in which the N-terminal half of echinoderm microtubule-associated protein-like 4, also known as EML4, is fused to the intracellular kinase domain of ALK. Clinically, the presence of ALK fusions is associated with EGFR TKI resistance. ALK gene rearrangement predicts response to inhibitors of the chimeric tyrosine kinase synthesized by this oncogene and could therefore assist in the management of this patient.

Epidermal Growth Factor Receptor, also known as EGFR, is a growth promoting protein lying within the cytoplasmic membrane. Its external domain binds growth factors and is the target of monoclonal antibody drugs while the internal domain, including the tyrosine kinase domain, is the target of small molecule drugs, known as tyrosine kinase inhibitors Fine needle aspirates, unstained slides and Formalin Fixed Paraffin Embedded tissues, when core tissue is obtained, can be sent for EGFR mutation analysis or increased gene copy number.

An established test for ALK gene rearrangement is fluorescence in situ hybridization, also known as FISH. Some molecular laboratories require at least 2 mL of special media for fine needle aspirates or 10% neutral buffered FFPE tissue; ALK gene rearrangement can also be detected by immunohistochemistry, PCR and DNA sequencing[2], and is feasible in EBUS-TBNA specimens. As of June, 2013, FISH is the only FDA-approved test in the United States.

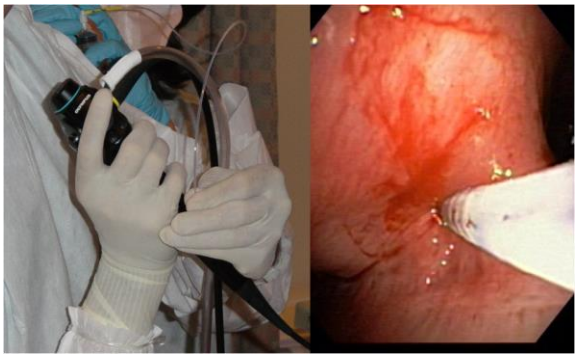
References:

1. Rosell R, Moran T, Queralt C, et al; Spanish Lung Cancer Group. Screening for epidermal growth factor receptor mutations in lung cancer. N Engl J Med. 2009;361:958-67.
2. Rosell R, Gervais R, Vergnenegre A, et al. Erlotinib versus chemotherapy (CT) in advanced non-small cell lung cancer (NSCLC) patients (p) with epidermal growth factor receptor (EGFR) mutations: Interim results of the European Erlotinib Versus Chemotherapy (EURTAC) phase III randomized trial. J Clin Oncol 29: 2011 (suppl; abstr 7503).
3. http://www.mycancergenome.org/molecular-pathology (accessed 8/23/11).
4. Nakajima T, Yasufuku K, Nakagawara A, et al. Multi-gene mutation analysis of metastatic lymph nodes in non-small cell lung cancer diagnosed by EBUS-TBNA. Chest 2011 Apr 28. [Epub ahead of print].
5. Sakairi Y, Nakajima T, Yasufuku K, et al. EML4-ALK fusion gene assessment using metastatic lymph node samples obtained by endobronchial ultrasound-guided transbronchial needle aspiration. Clin Cancer Res. 2010;16:4938-45.

[2] As of this writing, the only FDA approved test in the United States is FISH.

The Case Continues

After obtaining all the specimens and sending them to the appropriate laboratories, including for all three molecular analysis tests, the procedure was ended. The patient was transferred to the recovery area and discharged home. On follow-up visit, the final results of the procedure were discussed. The patient had primary lung adenocarcinoma, stage IIIB. EGFR mutation analysis showed deletion of exon 19; KRAS mutation was negative. ALK rearrangement was negative by FISH.



Question 6: In case of personalized therapy, this patient will most likely benefit from which of the following?

A. ALK-directed therapy
B. EGFR-TKI
C. Folate analogue metabolic inhibitors

Answer: B

For patients with advanced NSCLC whose tumors harbor ALK rearrangement, there is evidence of a benefit from ALK TKI treatment. Our patient, however, tested negative for ALK rearrangement.

EGFR TKIs administered to Asian patients who were EGFR mutation positive improved progression free survival when compared with those who received chemotherapy with carboplatin/paclitaxel. The response rate in mutation positive patients was 73.7% versus 30.7 % in the chemotherapy group. Progression free survival was 10.8 months versus 5.4 months, favoring EGFR TKIs.

Folate analogue metabolic inhibitors have been studied in NSCLC patients with low levels of thymidylate synthase, also known as TS, expression. This enzyme involved in DNA biosynthesis is responsible for maintaining intracellular levels of thymidine, important for DNA synthesis and repair. Immunohistochemistry and RT-PCR on FFPE tissue can be analyzed for TS expression, which is higher in patients with squamous cell carcinoma compared to adenocarcinoma and is correlated with resistance to Folate analogue metabolic inhibitors.

In Caucasians, a 58% objective response rate was reported using EGFR TKIs compared with 15% response after chemotherapy. Progression free survival improved from 5.2 months to 9.4 months. Because our patient tested positive for EGFR mutation, initiation of EGFR TKI is appropriate.

References:

1. Kwak EL, Bang YJ, Camidge DR, et al. Anaplastic lymphoma kinase inhibition in non-small-cell lung cancer. N Engl J Med 2010; 363:1693-1703.
2. Mok TS, Wu YL, Thongprasert S, et al. Gefitinib or carboplatin-paclitaxel in pulmonary adenocarcinoma. N Engl J Med. 2009;361: 947-57.
3. Maemondo M, Inoue A, Kobayashi K, et al; North-East Japan Study Group. Gefitinib or chemotherapy for non-small-cell lung cancer with mutated EGFR. N Engl J Med. 2010;362:2380-8.
4. Rosell, R. Gervais R, Vergnenegre A, et al. Erlotinib versus chemotherapy (CT) in advanced non-small cell lung cancer (NSCLC) patients (p) with epidermal growth factor receptor (EGFR) mutations: Interim results of the European Erlotinib Versus Chemotherapy (EURTAC) phase III randomized trial. J Clin Oncol 29: 2011 (suppl; abstr 7503).
5. Scagliotti GV, Parikh P, von Pawel J, et al. Phase III study comparing cisplatin plus gemcitabine with cisplatin plus pemetrexed in chemotherapy-naive patients with advanced-stage non-small-cell lung cancer. J Clin Oncol 2008; 26:3543-3551.

The Case Continues

The patient elected to have biomarker-directed therapy rather than chemoradiation. She was started on EGFR TKI targeted therapy. She tolerated treatment well except for mild diarrhea and an acne type rash over her neck and face which disappeared after several weeks of treatment. Her follow up PET CT scans were stable until 12 months later when she showed increased lymphadenopathy size and activity in stations 4R, 4L and 11L. She reported intermittent hemoptysis, but remained active and independent in her activities of daily living. She had strong social support and wanted additional therapy. This is discussed at your multidisciplinary lung cancer conference.

Question 7: What do you recommend next?

A. Chemotherapy with carboplatin and taxol
B. Confirm progression of disease before recommending additional therapy
C. Consult palliative care
D. Combined chemoradiation

Answer: B

Initiating chemotherapy or palliative care only without confirming progression of disease is probably undesirable. It is also noteworthy that some patients may have a granulomatous disorder that can mimic cancer. Patients may also have tumor markers, such as over expression of ERCC1, which predicts resistance to platinum based chemotherapy. Consulting palliative care medicine is appropriate, particularly because progressive disease is a concern, but may not be the next immediate step in this patient's management because her quality of life and activities of daily living have not deteriorated.

In this patient with lung adenocarcinoma and history of EGFR mutation, repeat biopsy is done to confirm disease progression and to evaluate for secondary mutations which might offer prognostic value and help guide referral towards clinical trials.

Patients with a diagnosis of cancer and evidence of mediastinal and or hilar lymphadenopathy on PET CT should undergo a tissue sampling procedure to avoid inaccurate upstaging and inappropriate therapeutic management.

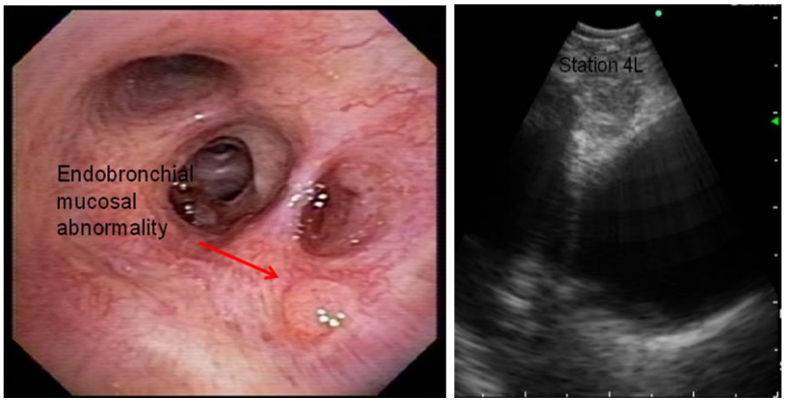
References:

1. Sequist LV, et al. Genotypic and histological evolution of lung cancers acquiring resistance to EGFR inhibitors. Sci Transl Med 2011;3:75ra26.
2. Kennedy M, Jimenez C, Mhatre A, et al. Clinical implications of granulomatous inflammation detected by endobronchial ultrasound transbronchial needle aspiration in patients with suspected cancer recurrence in the mediastinum. J Cardiothorac Surg. 2008; 3:8.
3. Olaussen KA, Dunant A, Fouret P, et al. DNA repair by ERCC1 in non-small-cell lung cancer and cisplatin-based adjuvant chemotherapy. N Engl J Med. 2006;355:983-991.

The Case Continues

The patient is discussed in the multidisciplinary chest conference. A decision is made to proceed with bronchoscopy in order to evaluate for possible airway involvement with tumor given the patient's new onset of hemoptysis, and to perform EBUS-TBNA in an attempt to confirm progression of disease within the mediastinum given the suspected progression of disease on PET-CT. Bronchoscopy revealed

mucosal abnormalities within the right bronchus intermedius and EBUS imaging confirmed the presence of mediastinal and hilar lymphadenopathy.



Question 8: What would you do next?

A. Endobronchial biopsy, lavage and brushings of the abnormality in the bronchus intermedius.
B. Sequential EBUS-TBNA from stations 11L, 4L and 4R
C. Sequential EBUS-TBNA from stations 4L, 4R, and 11L, stopping should any of these nodal stations be positive.
D. Endobronchial sampling by biopsy, lavage, and brushing in addition to sequential EBUS-TBNA from stations 11L, 4L, and 4R.

Answer: D

For central lesions such as those visible during flexible bronchoscopy, the diagnostic yield of endobronchial biopsy is 74% as compared to 46% for peripheral lesions. To achieve 90% probability of positive biopsy for malignancy, 5 samples are usually required for visible tumor. For molecular markers analysis, endobronchial and trans-thoracic biopsies provide adequate tissue for DNA sequencing in 89% of samples.

On the other hand, for cytological diagnosis, the diagnostic yield of bronchial brushings in central lesions is 59% compared to about 46% for peripheral lesions. The utility for molecular markers analysis has not been clearly determined.

Bronchioloalveolar lavage has a diagnostic yield of 48% for central lesions and 43% for peripheral lesions. There is emerging data on the use of lavage for molecular analysis but testing a major limitation relates to tumor cellularity.

EBUS-TBNA from abnormal mediastinal/hilar nodes in patients with known or highly suspected lung cancer has a diagnostic yield for malignancy of 94% with usually at least 3 specimens per nodal

station being obtained for diagnosis if ROSE is not utilized; two aspirations per nodal station are considered acceptable when at least one core obtained by first two specimens. For molecular markers, adequacy of EBUS-TBNA specimens varies in the published literature between 72-99%.

In this patient combined sampling techniques are warranted. Using EBUS-guided TBNA, left sided nodes should be sampled first since the patient's primary tumor was on the right. A reasonable approach, therefore, would be to start with the newly PET positive 11L node followed by sampling nodes at level 4L and potentially 4R.

Published literature supports a practice of combined techniques to increase the yield for central lesions for which the diagnostic yield is 88% versus 69% for peripheral lesions. Brushing and washing increases diagnostic yield by up to 17%.

Because the utility of bronchoscopic specimens for molecular markers analysis is based on number of intact malignant cells provided for analysis, and because specimens with malignant cells in suspension can be prepared in cell block for immunohistochemistry or submitted for molecular testing, a combination of endobronchial techniques and EBUS-TBNA techniques seems to be the most advantageous in terms of specimen acquisition for molecular analysis.

References:

1. British Thoracic Society guidelines on diagnostic flexible bronchoscopy Thorax 2001;56:(suppl I) i1–i21.
2. Arcilla M, et al. Rebiopsy of Lung Cancer Patients with Acquired Resistance to EGFR Inhibitors and Enhanced Detection of the T790M Mutation Using a Locked Nucleic Acid-Based Assay Clin Cancer Res 2011;17:1169-1180.
3. Van der Drift M, et al. The Additional Value of the Ras-Association Domain Family 1A Gene Methylation and Kirsten Rat Sarcoma 2 Viral Oncogene Homolog Mutation Analyses in Washings in Nondiagnostic Bronchoscopy Chest 2012;141;169-175.
4. Tanner NT, et al. Utilizing Endobronchial Ultrasound With Fine-Needle Aspiration to Obtain Tissue for Molecular Analysis J Bronchol Intervent Pulmonol Volume 18, Number 4, October 2011.

The Case Continues

Five endobronchial biopsies were performed from the airway lesion. Small tissue fragments were fixed in formalin 10% and sent to pathology to prepare formalin fixed paraffin embedded tissues, also known as FFPE. Five EBUS-guided TBNA aspirates were obtained performed from nodal station 11L. Three slides were air dried and stained with Romanowsky solution for rapid on site evaluation which showed the presence of malignant cells consistent with NSCLC. Three slides were fixed in alcohol and sent to the pathology laboratory for Papanicolau staining. Aspirates were also placed in Cytolyt solution for cell block preparation.

Question 9: What would you do next if the patient wants to be considered for clinical trials using targeted therapy?

A. Discuss the adequacy of the specimen with your pathologist on-site prior to requesting molecular tests.
B. Given the scant amount of tissue, order molecular testing without confirming non squamous non small cell carcinoma by immunohistochemistry.
C. Wait for final results. After malignancy is confirmed, refer the patient for chemotherapy without ordering molecular testing.
D. Wait for final results. After malignancy is confirmed, initiate discussions about quality of life and refer the patient to a palliative care specialist.

Answer: A

It is important to assure that samples contain adequate cellularity and representative tissue for analysis. Care should be taken to avoid wasting tissue on unnecessary IHC studies, and to conserve as much tissue as possible for molecular analysis when indicated. Because the literature suggests that specimens should contain at least 50-70% tumor cells for mutation analysis, sending specimens directly for molecular testing without a pathologist's review for quality and quantity is not recommended.

In this patient, specimens were available for analyzing markers predictive of response and or resistance to certain chemotherapy agents and molecular targets. Evidence suggests that high expression of thymidylate synthase, for example, could predict resistance to pemetrexed. Initiating chemotherapy without performing molecular testing may not be cost-effective.

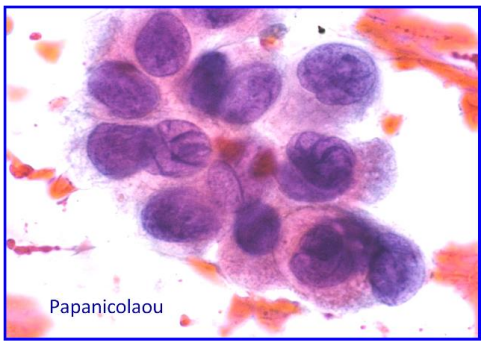
Operators collecting small specimens should carefully use appropriate smear techniques, attempt to obtain tissue cores, and communicate on-site with their pathologists to assure sample adequacy and appropriate processing.

References:

1. Pirker R, et al. Consensus for EGFR mutation testing in non-small cell lung cancer: results from a European workshop. Thorac Oncol. 2010 Oct;5(10):1706-13
2. Takezawa K. Identification of thymidylate synthase as a potential therapeutic target for lung cancer et al. British Journal of Cancer (2010)

The Case Continues:

Papanicolau stains were positive for malignancy, most likely adenocarcinoma. Both endobronchial biopsy and EBUS-TBNA specimens were analyzed by the cytopathologist and considered adequate for molecular analysis. IHC confirmed adenocarcinoma. TTF1 was positive and P63 was negative. Given the patient's history of primary lung malignancy and no clinical suspicion for extrathoracic malignancy metastatic to the lung, specimens were not processed for further Immunohistochemistry.



Courtesy M. Ferretti, with permission

Question 10: Which molecular markers would you test for now?

A. ERCC1, RRM 1 and TS
B. T790M mutation
C. MET amplification
D. All of the above

Answer: D

While data regarding clinical use is still emerging, all markers listed above could impact management. Testing for these markers is relevant if clinical trials are accessible to the patient.

ERCC1, RRM1 and TS could predict response /and or resistance to certain chemotherapy agents. ERCC1 is an important component of nucleoside excision repair. Because platinum-based chemotherapy works by creating platinum-DNA adducts, increased levels of ERCC1 indicates resistance to platinum based therapy while low levels indicate sensitivity. Regulatory subunit of ribonucleotide reductase, also known as RRM1, is the target of gemcitabine. In some studies, high levels are associated with gemcitabine resistance and poor outcome. High levels of TS indicate pemetrexed resistance, but levels are usually higher in squamous cell compared to adenocarcinoma, which may be why squamous cell carcinoma does not respond to pemetrexed.

In patients treated with TK inhibitors for EGFR positive lung cancer, adaptive resistance commonly develops during therapy. Mechanisms of resistance include T790M mutation and MET amplification. If tested positive, this patient could be enrolled in clinical trials directed towards these molecular markers.

References:

1. Olaussen et al: DNA Repair by ERCC1 in Non–Small-Cell Lung Cancer and Cisplatin-Based Adjuvant Chemotherapy. N Engl J Med. 2006;355:983-991.
2. Rosell R et al: Ribonucleotide reductase mRNA expression and survival in gemcitabine/cisplatin-treated advanced non-small-cell lung cancer patients. Clin Cancer Res 10:1318-1325, 2004.
3. Kobayashi S et al. EGFR mutation and resistance of non-small-cell lung cancer to gefitinib. N Engl J Med. 2005;352(8):786.
4. Cipriani NA et al. MET as a target for treatment of chest tumors. Lung Cancer. 2009;63(2):169.

Case conclusion:

T790M mutation was positive and MET was not amplified. ERCC1 and TS levels were low. RRM1 was also low. The patient wanted to receive standard chemotherapy. She refused enrollment in a clinical trial. Given her tumor's low expression of ERCC1 and TS, she is more than likely to respond to a combination of platinum and Folate analogue metabolic inhibitors such as pemetrexed.

The patient was referred to a palliative care specialist in consultation only. By integrating palliative care consultation, the multidisciplinary team felt the patient's overall quality of life might be improved. Studies show a benefit of integrating palliative care with oncologic care. Both chemotherapy and performance status have been shown to positively impact survival in patients with advanced NSCLC.

Literature shows that the early introduction of palliative care prolongs survival among patients with advanced III B and IV non-small-cell lung cancer stage.

References:

1. Olaussen et al. DNA Repair by ERCC1 in Non–Small-Cell Lung Cancer and Cisplatin-Based Adjuvant Chemotherapy. N Engl J Med. 2006;355:983-991
2. Rosell R et al: Ribonucleotide reductase mRNA expression and survival in gemcitabine/cisplatin-treated advanced non-small-cell lung cancer patients. Clin Cancer Res 10:1318-1325, 2004.
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Clinical Stem 2

A patient with pulmonary nodules 1 year after curative intent resection of primary lung adenocarcinoma[3]

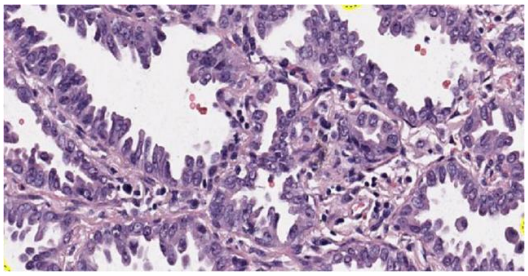
Learning objectives: the webcast participant will be able to:

1. Describe patient management strategies to assure adequate material is obtained from small samples for lung cancer diagnosis and molecular analysis.
2. Describe rationales for molecular testing for diagnosis or in case of rebiopsy.
3. Describe optimization strategies for acquisition and handling of small cytology and histology samples.
4. Describe laboratory processing requirements of small cytology and histology samples to ensure that sufficient material is available for molecular analysis.

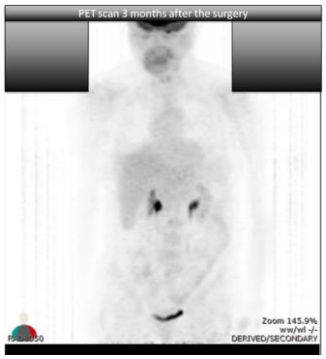
[3] Disclaimer: This is a fictitious clinical case scenario based on a conglomerate of real patient data, modified to avoid any possibility for patient identification and to help meet educational objectives. Any resemblance to real persons, living or deceased is purely coincidental.

Case Description

A 45 year-old, nonsmoking, white female diagnosed with stage II B primary NSCLC underwent right lower lobectomy. The tumor was adenocarcinoma with no visceral pleural extension. Surgical lymphadenectomy revealed no peribronchial, hilar or mediastinal lymph node involvement. The tumor was staged T3N0M0. Adjuvant chemotherapy was well tolerated except for fatigue, nausea, constipation and transient peripheral neuropathy. A surveillance PET-CT scan obtained after completion of adjuvant chemotherapy showed no residual tumor.



LUNG ADENOCARCINOMA



Clinical Stem

Question 1. Would you have ordered a surveillance PET-CT scan after completion of adjuvant chemotherapy?

A. Yes, because a high level of evidence demonstrates that surveillance PET-CT after curative intent surgery improves survival.
B. Yes, because surveillance PET-CT detects more tumor recurrence events than conventional imaging such as whole body CT, bone scintigraphy and brain MRI combined.
C. Yes, but this is debatable. This particular patient has a high risk for recurrence. If detected at an early stage, she may benefit from curative intent treatment.
D. Yes, because almost all recurrences occur within the first 6 months after curative surgery.

Answer: C

This patient with pathologic stage IIB has a higher risk of recurrence than if she had limited IA disease. A goal of surveillance imaging is to detect lung cancer recurrence or second primary lung cancer early enough to warrant curative retreatment. Studies to date do not show that surveillance PET-CT improves overall survival. Current ACCP, NCCN, ESMO and NICE guidelines do not recommend PET-CT for routine surveillance after curative intent treatment, but PET-CT was shown to be useful for restaging patients after adjuvant therapy.

Several studies, including one randomized trial, show that PET-CT after resection leads to earlier detection of a greater number of asymptomatic recurrences compared to more traditional imaging, although many false positive findings are detected.

Recurrences of NSCLC during the first four years after curative intent resection seem to gather at specific times. There is an initial surge 9 months after surgery, followed by two smaller surges at the end of 2 and 4 years, respectively. These recurrences may not be detected by regularly scheduled imaging studies.

Surveillance radiologic imaging recommendation strategies do not currently take into account prognostic factors associated with recurrence risk. These factors are relevant to designing a personalized surveillance strategy as part of a patient-centric approach to lung cancer diagnosis and treatment.

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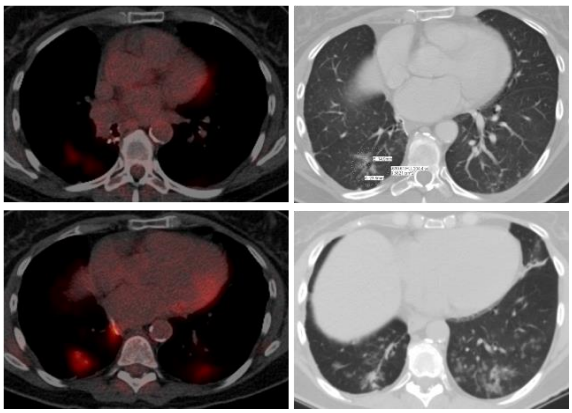
The Case Continues

The patient did well until her next clinical examination six months later. This was one year after surgical resection. She had no symptoms, but PET-CT scan now showed multiple small hyper-metabolic bilateral pulmonary parenchymal lesions. There were no suspicious extra-thoracic FDG avid abnormalities. The patient's history and imaging studies were presented at a weekly multidisciplinary lung cancer conference.



Question 2. What patient management strategy would you suggest?

A. Continue clinical follow-up and surveillance imaging because lesions are likely inflammatory
B. Proceed with bronchoscopy or CT-guided needle aspiration for tissue diagnosis
C. Proceed with bronchoscopy or CT-guided needle aspiration for tissue diagnosis and molecular analysis.
D. Consult thoracic surgery for VATS

Answer: C

Multidisciplinary lung cancer teams ideally include representatives from pulmonary medicine, thoracic surgery, medical and radiation oncology, palliative care, radiology, and pathology.

When findings on PET-CT scan appear inflammatory, continued clinical and radiologic surveillance may avoid unnecessary procedures in patients with benign lesions, but delay diagnosis and treatment in case of malignancy.

Tissue is needed to confirm a diagnosis of advanced lung cancer and to individualize treatment based on genetic alterations such as sensitizing EGFR mutations or EML-ALK fusion genes.

Safe and cost-effective strategies to obtain adequate tissue for diagnosis and molecular analysis are dictated by patient-related factors and lesion characteristics.

Electromagnetic navigation bronchoscopy, also known as ENB, combines simultaneous CT virtual bronchoscopy with real-time flexible bronchoscopy. It has an overall diagnostic yield of 70% and pneumothorax rate of approximately 3%. If available, ENB is a reasonable first option to obtain diagnostic tissue from peripheral lesions, even when their diameter is less than 2 cm. The yield is increased to 80% if an airway is seen leading to the lesion.

Radial probe endobronchial ultrasonography, known as REBUS, is used to obtain tissue samples from peripheral lung lesions, even those too small to be visualized using fluoroscopy. Sensitivity is 0.73 for detecting lung cancer, with a mean positive likelihood ratio of 26 and a negative likelihood ratio of 0.28. REBUS-guided

TBNA increases yield from 46% to 69% compared with TBNA without REBUS in nodules less than 2 cm in diameter.

Conventional bronchoscopic lung biopsy for peripheral lesions less than or equal to 2 cm has a diagnostic yield as low as 14%. Bronchoscopy with bronchioloalveolar lavage might be performed to identify infectious etiologies.

The diagnostic yield of CT-guided needle aspiration and biopsy varies between 36% and 84%. Pneumothorax requiring chest tube drainage is reported in 5-10% of procedures. Risk factors for pneumothorax include surrounding emphysema, the lesion's proximity to fissures, and needle insertion through aerated lung parenchyma.

Video Assisted Thoracic Surgery, also known as VATS, has a sensitivity and specificity approaching 100%, but its associated mortality is approximately 1%. VATS may be appropriate in patients who are surgical candidates. This patient, however, would not be a surgical candidate if the bilateral lesions are confirmed to represent stage IV recurrent lung cancer.

In patients with confirmed or suspected lung cancer, results from a multidisciplinary conference may lead to improved outcomes, less fragmented care, fewer delays in treatment, more structured coordination of care, and improved patient satisfaction, especially when multimodality treatment is being considered.

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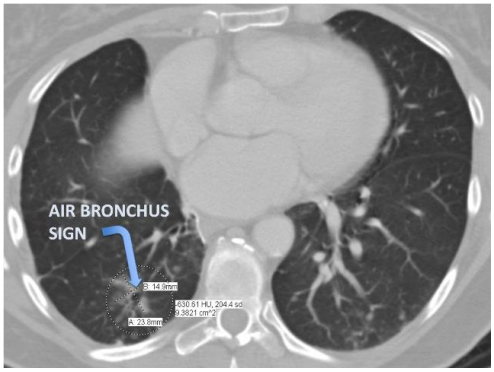
The Case Continues

The multidisciplinary conference discussion focused on performing bronchoscopy or CT-guided needle aspiration biopsy to obtain adequate material for histologic diagnosis and molecular markers. The radiologist identified an air bronchus sign leading to the right peripheral nodule. The Oncologist said that results from

molecular analyses such as EGFR mutation and ALK translocation might alter therapeutic management in case of proven lung cancer recurrence. Based on existing literature and team experience, a bronchoscopic approach was recommended.



Question 3. Which of the following bronchoscopic procedures are most likely to obtain adequate samples for diagnosis and molecular analysis from the small peripheral lesion?

A. Bronchoscopy with radial probe endobronchial ultrasonography
B. Bronchoscopy with electromagnetic navigation
C. Bronchoscopy with electromagnetic navigation and radial probe ultrasonography

Answer: C

In one multicenter prospective, randomized controlled trial the diagnostic yield of combined REBUS with ENB was 88%, significantly better than either REBUS or ENB alone. In the combined REBUS and ENB group, navigation to the lesion was first performed using the ENB system. When the lesion was reached, the ENB sensor probe was removed and the REBUS probe was inserted through the extended working channel of the bronchoscope to confirm visualization of the target lesion.

Diagnostic sensitivity is increased when radial probe endobronchial ultrasound is combined with electromagnetic navigational bronchoscopy.

References

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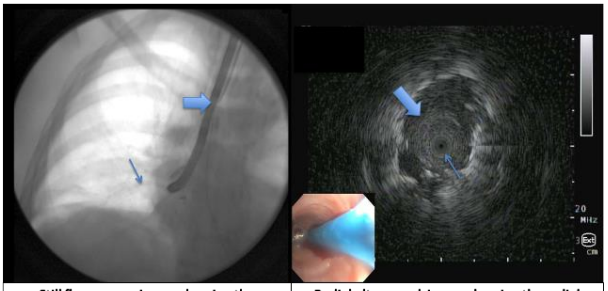
The Case Continues

After discussing the risks and benefits of bronchoscopic interventions with the patient and her family, a shared decision was made to perform Electromagnetic navigational bronchoscopy and radial probe EBUS to biopsy the peripheral lesion.



Still fluoroscopy image showing the extended working channel (thin arrow) after navigation and the flexible bronchoscope (thick arrow). The nodule (target) is not seen on fluoroscopy	Radial ultrasound image showing the radial probe (thin arrow) inside the pulmonary nodule (thick arrow). The extended working channel (guide sheath) is visualized on white light bronchoscopy (left lower corner)

Question 4. How many REBUS-guided biopsies would you obtain to assure adequate tissue for histologic diagnosis AND sufficient material for molecular analysis of EGFR mutation and ALK translocation

A. 1-3 biopsies
B. 4-5 biopsies
C. 6-10 biopsies

Answer: B

For EGFR tests, expert consensus currently suggests performing 4-5 biopsies to obtain more than 300 cells per biopsy. For ALK translocation testing using FISH, more than 100 assessable tumor cell nuclei are recommended.

Bronchoscopic forceps biopsies provide sufficient tissue for diagnosis and molecular analysis. Significantly larger biopsies and artifact-free tissue specimens are reported after cryobiopsy of endobronchial lesions.

Regardless of how they are obtained, biopsy specimens should be immediately fixed in an adequate amount of neutral buffered 10 % formalin, usually a ratio of 5–10 to biopsy volume, and embedded in paraffin, creating a Formalin Fixed Paraffin Embedded tissue, also known as FFPE. A fixation time of 6 to 12 hours for small biopsy samples is considered optimal.

Before samples are sent for molecular analysis, the specimen's tumor cell content should be assessed by the pathologist to enhance the reliability of subsequent molecular test results.

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The Case Continues

Five biopsies were obtained without complications. The bronchoscopy assistant asks whether brushings, lavage and TBNA should also be performed.

Question 5. Are cytology specimens from bronchioloalveolar lavage fluid and brushings satisfactory for molecular testing?

A. Yes, there is strong evidence proving specimen adequacy for molecular testing
B. No, these specimens are sufficient only for cytomorphologic diagnosis
C. This question has not been answered for all molecular markers

Answer: C

Bronchoscopic lavage and brushing specimens from peripheral lung lesions provide a cytomorphologic diagnosis of malignancy in approximately 60% and 45% of cases, respectively. The yield is less for lesions less than 3 cm in diameter. Brushings are the only source of diagnosis in approximately 5% of cases. BAL fluid processing has been standardized. A combination of techniques is indicated to increase diagnostic yield.

Accuracy of definitive cytomorphologic diagnosis is 96%, although in studies, brushings and lavage specimens are typically under-represented compared with fine needle aspirations or pleural fluid. Overall accuracy of cytologic tumor subtyping in concordance with histology is 93%.

There are concerns that low cellularity in exfoliative cytology samples such as sputum, bronchial washes, brushings, and lavage may not provide adequate material for molecular testing, but any cytology specimen with cellular material in suspension can be processed and saved as a paraffin embedded cell pellet, also known as a cell block.

Cytologic specimens from fine needle aspiration, pleural fluid, bronchial washing, brushing and bronchoalveolar lavage are suitable for EGFR and KRAS sequencing. In the case of EFGR, sensitivity for mutation detection is comparable to that of surgical specimens.

Considering the rapidly evolving role and growing number of molecular markers relevant to lung cancer patient management, the adequacy of cytology samples should be determined on an individual basis by a cytopathologist. Similar to surgical specimens, cell blocks are used for immunohistochemistry or molecular testing processing.

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1. Mazzone P, Jain P, Arroliga AC, Matthay RA. Bronchoscopy and needle biopsy techniques for diagnosis and staging of lung cancer. Clin Chest Med. 2002; 23: 137-58.
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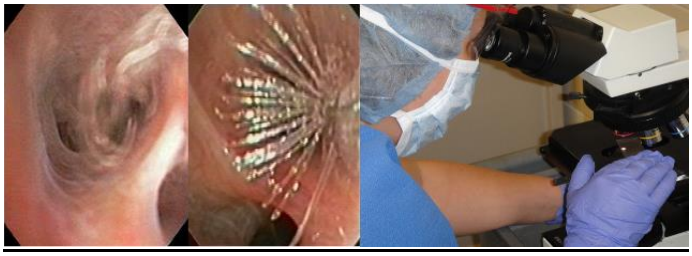
The Case Continues

BAL and two brushings were performed from the target lesion. According to institutional policy, the pathologist was called to the procedure suite and a procedure note was written immediately after the intervention. The bronchoscopist also called the referring oncologist to discuss findings.



Question 6. Which of the following elements help assure specimen adequacy for effective molecular analysis?

A. Document sample type, such as cytology or tissue biopsy, how it was obtained, and the location from where it was obtained.
B. Document the date and time of sample acquisition
C. Inform pathologists that a diagnosis of lung cancer or lung cancer recurrence is suspected so that a limited number of immunohistochemistry stains (IHC), are used to determine site of origin
D. Inform pathologists to proceed with molecular analysis only after histologic confirmation of malignancy
E. All of the above

Answer: E

Clear communication with the pathologist and treating physician helps assure appropriate handling of small samples in the pathology laboratory. Accurate and relevant clinical information include a description of sample site and type, clinical suspicion for primary and recurrent lung cancer versus metastatic disease, history of previous cancers, relevant history of prior surgical, oncologic or radiation therapy and need for molecular analysis in case of non-squamous, non-small cell lung cancer.

The pathologist should anticipate the appropriate use of IHC stains and molecular analysis to avoid wasting tissue unnecessarily for tests that are not required in the clinical situation.

Documenting the time of specimen acquisition is crucial for calculating time to specimen fixation. While fixation of lung cancer tissue has not been standardized, short fixation times of 6–12 hours for small biopsy specimens and 8–18 hours for larger resection specimens in 10% neutral buffered formalin are optimal for DNA and RNA-based tests, as well as FISH assays.

Samples should be examined by a pathologist to document the tumor's cellular content and purity in the area of tissue being sent for molecular analysis. Sample assessment is critical to obtain accurate results and to prevent false negatives.

An ideal sample has a high proportion of malignant cells relative to benign cells, and a low amount of substances such as mucin or necrotic tissue that may inhibit amplification.

Preserving scant tissue for most relevant tests is a major challenge facing pathologists who handle small volume cytology and histology specimens

Sending specimens directly to the molecular laboratory without prior assessment of tumor content by a pathologist should be avoided.

References:

1. Thunnissen E, Kerr KM, Herth FJ, et al. The challenge of NSCLC diagnosis and predictive analysis on small samples. Practical approach of a working group. Lung Cancer 2012; 76: 1-18.
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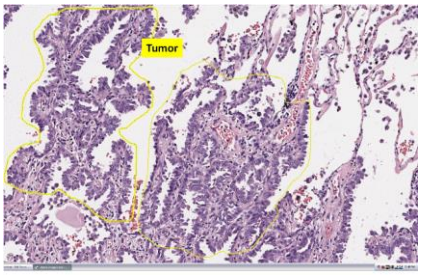
The Case Continues

Biopsy specimens and brushings showed adenocarcinoma. BAL was non diagnostic. The biopsy specimen was adequate for EGFR mutation analysis but not for ALK FISH testing because of insufficient number of accessible tumor nuclei.



Question 7. What is the most appropriate next step?

A. Consult thoracic surgery for VATS to obtain a better specimen
B. Initiate chemotherapy
C. Request analysis of molecular markers from the original resected specimen

D. Wait for results of EGFR testing from the repeat biopsy specimen, perform ALK testing, and request original resected specimens for analysis of molecular markers.

Answer: D

Performing VATS to obtain more tissue or initiating chemotherapy while waiting for results from the current biopsy in this patient is counter-intuitive. Because EGFR status in primary and metastatic tumors may not be identical, however, EGFR status in the primary tumor may not predict EGFR status in metastases or sites of disease recurrence.

ALK gene rearrangements are currently detectable using immunohistochemistry, FISH, or reverse-transcriptase polymerase chain reaction, also known as RT-PCR. As of June 2013 in the United States, the only FDA-approved test for detection of ALK rearrangements is the FISH test.

Molecular testing on resected stage I-III lung cancer specimens allows for enrollment in clinical trials that target mutation-specific, directed therapy and assists with therapy selection for recurrent disease when it occurs. Resected surgical specimens are the gold standard against which small volume histology or cytology specimens are measured for adequacy, both for histologic diagnosis of cancer and molecular testing. Resected tissue may be available, however, in only that subset of patients with NSCLC who undergo surgical resection with curative intent.

While the most recent available tissue is preferred for molecular analysis there is no strong evidence to justify procedures solely to procure tissue from a metastasis prior to initiation of TKI therapy if an earlier primary lesion is available and suitable for analysis, unless there is strong suspicion of its origin from a separate primary.

Guidelines from the College of American Pathologists state that in the absence of previous or current therapy with a target inhibitor, primary tumors and metastatic lesions are equally suitable for testing.

The choice of which sample to test should be based mainly on the sample's quality characteristics such as tumor content and preservation, rather than on whether it is from a primary or metastatic lesion.

References:

1. Thunnissen E, Bubendorf L, Dietel M, et al. EML4-ALK testing in Non small cell carcinomas of the lung: A review with recommendations.Virchows Arch 2012;461: 245-257.
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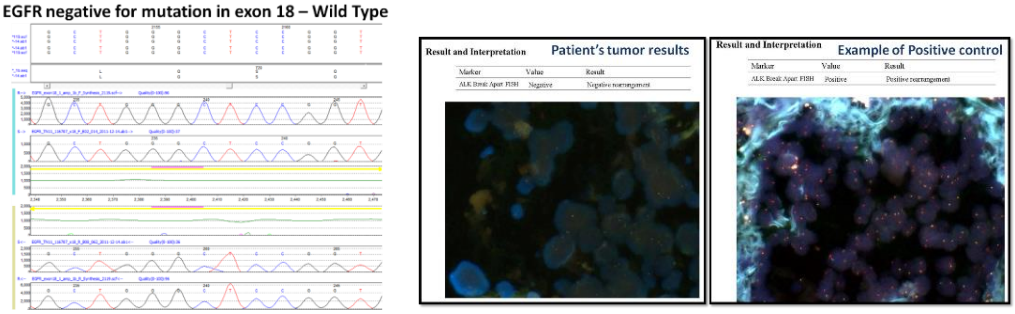
The Case Continues

EGFR mutation testing of the repeat biopsy was negative. The initially resected specimen was analyzed retroactively. It was EGFR, KRAS, and ALK FISH negative



Question 8. What do you recommend now?

A. Best supportive care
B. Platinum-based chemotherapy

Answer: B

Platinum-based chemotherapy with or without Bevacizumab is recommended for patients with a good performance status. It is probably warranted given this patient's diagnosis of adenocarcinoma, even though she has advanced disease. Best supportive care might be more appropriate if her performance status was poor.

A conversation with the patient and family members is warranted to discuss (1) goals of care, (2) quality of life concerns, (3) risk-benefit of therapeutic alternatives, and (4) possible enrollment in a clinical trial.

References:

1. Peters S, Adjei AA, Gridelli C, Reck M, Kerr K, Felip E; ESMO Guidelines Working Group. Metastatic non-small-cell lung cancer (NSCLC): ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up. Ann Oncol. 2012 Oct;23 Suppl 7:vii56-64.
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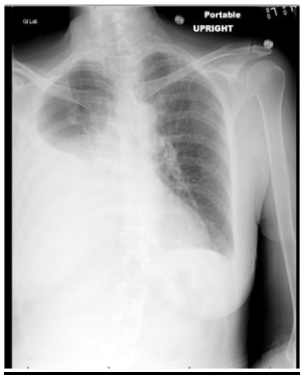
The Case Continues

Chemotherapy was initiated. The patient's performance status deteriorated significantly. Her ECOG score was 4. Three months after initiating treatment she complained of increasing shortness of breath and fatigue interfering with activities of daily living. Her chest radiograph showed a large right-sided pleural effusion. Thoracentesis removed 1 liter of serosanguinous fluid. The patient's dyspnea improved and she requested additional treatment. The lung was fully expanded on the post-procedure chest radiograph. Cytology was positive for adenocarcinoma, consistent with the lung primary. In view of evidence for progressive disease, pleural fluid was sent for molecular analysis.



Question 9. Was it appropriate to send pleural fluid for molecular analysis?

A. Yes. Pleural fluid is adequate for molecular analysis, and molecular analysis should be considered in case of disease progression because trials of novel agents against new molecular targets are available.
B. No. Pleural fluid samples are usually inadequate for molecular analysis.

Answer: A

The role of molecular markers is evolving rapidly. In this patient, the previously performed analyses did not investigate for other potentially targetable mutations relevant to enrollment in clinical trials. Many experts would argue for repeat testing, especially as more molecular markers are discovered and other therapeutic agents identified for patients harboring mutations.

Documenting results of molecular tests on body fluids is increasingly relevant. Studies show that cell blocks from pleural or pericardial fluid are adequate for EGFR and KRAS mutation analysis.

Similar to fine needle aspirates from other sites, pleural fluid cytology specimens qualifying for molecular analysis should contain at least 40% tumor cells. One semi-quantitative estimate of specimen cellularity describes sparse cellularity as less than 300 tumor cells, low cellularity as 300-1000 tumor cells, and normal cellularity as more than 1000 tumor cells. Specimens with sparse cellularity were shown to have a high PCR failure rate due to poor quality, or insufficient quantity of DNA quantity EGFR and KRAS mutation analysis.

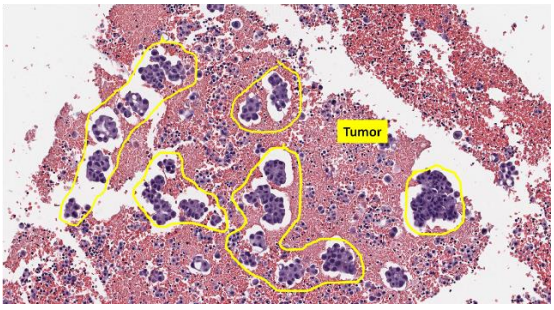
Pleural and pericardial fluids are inadequate for molecular testing in almost 4% of cases, which is less than the 7.5% insufficiency rate noted in CT-guided fine needle aspirates.

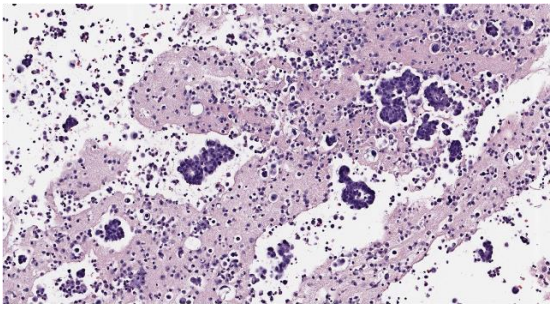
References

1. Billah S, Stewart J, Staerkel G, et al. EGFR and KRAS mutations in lung carcinoma: molecular testing by using cytology specimens. Cancer Cytopathol. 2011 ;119:111-7.
2. Rekhtman N, Brandt SM, Sigel CS, et al. Suitability of thoracic cytology for new therapeutic paradigms in non-small cell lung carcinoma: high accuracy of tumor subtyping and feasibility of EGFR and KRAS molecular testing. J Thorac Oncol. 2011;6:451-8.
3. Aisner DL, Marshall CB. Molecular pathology of non-small cell lung cancer: a practical guide. Am J Clin Pathol. 2012; 138: 332-46.

The Case Continues

Results of molecular analysis from the pleural fluid cell block were unchanged compared with the primary and metastatic lung parenchymal lesions. Three weeks after thoracentesis, the patient was hospitalized for recurrent dyspnea and right-sided effusion. In the absence of other findings, the recurrent effusion was felt to have caused her shortness of breath.





Low Expression: EGFR (Expression), ERCC1 High Expression: RRM1, TS
Wild-type: EGFR , EML4-ALK, KRAS

Question 10. What would you do now?

A. Repeat thoracentesis
B. Insert chest tube for talc slurry pleurodesis
C. Schedule pleurodesis by thoracoscopic talc insufflation
D. Insert a tunneled pleural catheter
E. Schedule a multidisciplinary conference to discuss alternatives

Answer: E

A multidisciplinary chest conference is probably the best next place to discuss optimal palliative strategies for this patient with a recurrent, symptomatic malignant pleural effusion.

Repeat thoracentesis is not warranted because of the rapid recurrence of the effusion and a likelihood this patient will live more than one month if her effusion and symptoms are controlled by other minimally invasive procedures

Rigid thoracoscopic or pleuroscopic talc insufflation pleurodesis is better than talc slurry, especially in patients with malignant effusions from lung and breast cancer. Respiratory complications including respiratory failure have been reported after insufflation and slurry.

Results from clinical trials show that tunneled pleural catheters, also known as TPCs, result in reduced post-procedure and overall length of stay compared with thoracoscopic talc insufflation or chest tube talc slurry pleurodesis. There are no differences in complication rates or in-hospital mortality. TPC placement may also be associated with significantly fewer ipsilateral re-interventions. TPCs are somewhat superior to talc slurry in terms of reliable drainage, pleurodesis and survival with effusion control but there may be no significant differences in quality of life between the two strategies.

Lung cancer patient management decisions take into account the patient's values, overall state of health, need for hospitalization, performance status, desire to return home, indications for hospice care, physician biases, preferences, experience and degrees of expertise as well as the patient's ability and informed consent to undergo minimally invasive procedures under local or general anesthesia.

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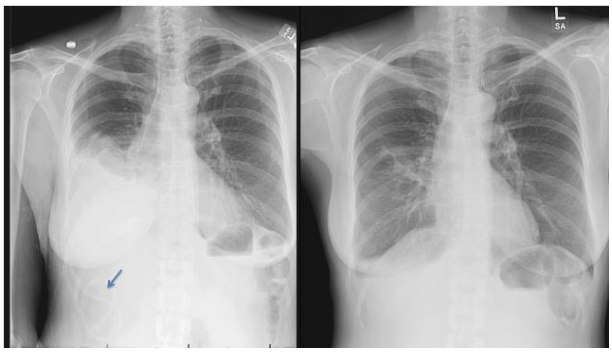
Case conclusion

The patient did not want to be hospitalized. She requested further systemic therapy if symptoms improved, or home hospice if they did not. A tunneled pleural catheter was therefore inserted. The procedure was performed and the patient was discharged home the same day. Dyspnea resolved and spontaneous pleurodesis was noted six weeks later. The indwelling catheter was removed during an outpatient follow-up visit. The patient's ECOG score had improved from 4 to 2. She preferred to travel and spend time with her family rather than be enrolled in a clinical trial.



Clinical Stem 3

A patient with right upper lobe adenocarcinoma progressing on biomarker-directed therapy[4]

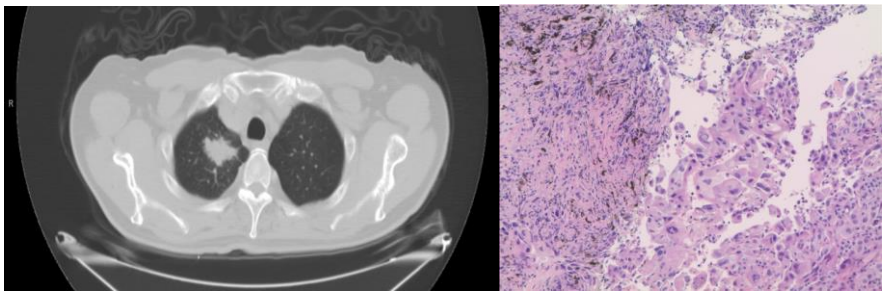
Learning objectives: the webcast participant will be able to:

1. Describe procedure-related strategy and planning for a patient with enlarging primary tumor and associated lymphadenopathy.
2. Describe techniques for overcoming challenges of obtaining and using small samples for molecular analysis.
3. Describe conservation strategies essential to providing adequate tissue for molecular analysis.
4. Describe acquisition and handling techniques to optimize specimen quality and adequacy for molecular testing.
5. Describe reasons for performing molecular analysis at time of diagnosis and disease progression.

[4] Disclaimer: This is a fictitious clinical case scenario based on a conglomerate of real patient data, modified to avoid any possibility for patient identification and to help meet educational objectives. Any resemblance to real persons, living or deceased is purely coincidental.

Case Description

A 62 year-old Asian male with a 20 pack-year smoking history presented to his primary care physician with persistent cough for 6 weeks. The chest radiograph showed a right upper lobe mass confirmed by chest computed tomography. There was no mediastinal lymphadenopathy. His FEV_1 was 36% of predicted. DLCO was 28% of predicted. Results from bronchoscopy with fluoroscopy-guided transbronchial lung biopsy showed poorly differentiated adenocarcinoma. Material was insufficient for molecular analysis due to the large number of immunostains performed in the pathology laboratory. The patient is discussed at your institution's multidisciplinary lung cancer conference.



Question 1: What would you recommend?

A. Refer the patient for lobectomy
B. Refer the patient for sub lobar resection
C. Refer the patient for stereotactic body radiation therapy (SBRT)
D. Proceed with integrated PET-CT for staging

Answer: D

Accurate staging is essential for managing patients with lung cancer because treatment options and prognosis differ significantly according to tumor stage. For this reason, patient findings are presented for discussion at multidisciplinary lung cancer meetings. If limited stage lung cancer is confirmed, treatment depends on patient operability. Systematic reviews conclude that CT scans have limited ability to rule in or exclude mediastinal metastasis. CT has a pooled sensitivity of 51% and specificity of 85% for identifying mediastinal lymph node metastasis.

PET is more accurate that Computed tomography. Pooled estimates of sensitivity and specificity for identifying mediastinal metastasis by PET scan are 74% and 85% respectively.

ACCP guidelines recommend PET scan to evaluate for potential mediastinal and extra-thoracic metastatic disease. PET should be considered in patients with clinical stage 1A lung cancer treated with curative intent (Grade of recommendation, 2C).

Reference

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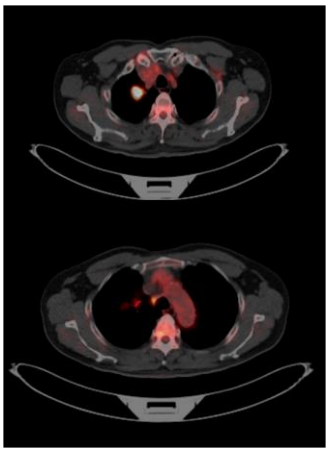
The Case Continues

Whole body integrated PET–CT showed high uptake in the primary tumor and in the mediastinum; lymph node station 4R.



Question 2: What do you recommend for mediastinal staging?

A. Surgery consult for mediastinoscopy or VATS
B. EBUS-guided TBNA. If non-diagnostic perform mediastinoscopy
C. EBUS and EUS-guided TBNA. If non-diagnostic consult surgery for mediastinoscopy or VATS

Answer: C

In patients with an abnormal PET scan, evaluation of the mediastinum with sampling of abnormal lymph nodes should be performed prior to surgical resection of the primary tumor. Results from a large, randomized trial show that a combined sonographic approach has greater sensitivity for detecting nodal metastases than mediastinoscopy alone. EBUS-TBNA can access mediastinal and hilar nodes with a diagnostic yield similar to mediastinoscopy. For patients with suspected nodal involvement, staging and diagnosis can be performed in a single setting.

Non-diagnostic EBUS or EUS-guided TBNA, defined as specimens that contain neither malignant cells nor lymphocytes, occurs in as many as 20% of individual aspirates and 10% of cases overall. Mediastinoscopy is likely warranted in these cases.

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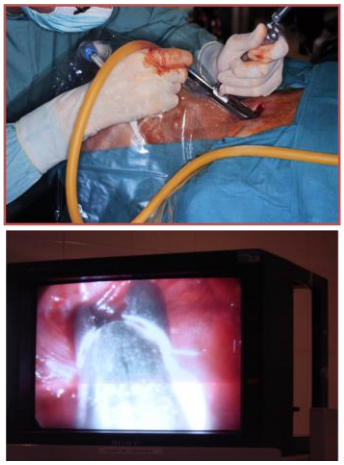
The Case Continues

Because sonography was not available, a surgical consultation was obtained and mediastinoscopy was performed. Lymph nodes at station 4R and 4L were positive for adenocarcinoma. The patient's tumor was staged T2a N3 M0 (stage IIIB).



Question 3: Would you send the lymph node specimens for molecular analysis?

A. Yes
B. No

Answer: A

Molecular analysis is warranted because this patient has locally advanced adenocarcinoma. The patient's previous primary tumor biopsy specimen was processed to the point that no material was left for molecular testing.

Studies demonstrate evidence of intra-tumoral heterogeneity with respect to mutations, and reports show discordance between the primary lesion and nodal metastases. It is not clear whether the potential for discordance should affect decisions on which specimen to test. In practice, biopsy of the most rapidly growing tumor is usually performed because it presumably contains the most biologically aggressive genetic alterations.The CAP/IASLC/AMP guideline states that either the primary or metastatic sites are acceptable for molecular testing.

Given the importance of acquiring sufficient and adequate tissue for molecular analysis, repeat bronchoscopic biopsy, or lymph node and tumor biopsies by mediastinoscopy or VATS may be warranted outside the staging setting.

References

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2. Gerlinger M, Rowan AJ, Horswell S et al. Intratumor heterogeneity and branched evolution revealed by multiregion sequencing. N Engl J Med. 2012; 366(10):883-92.
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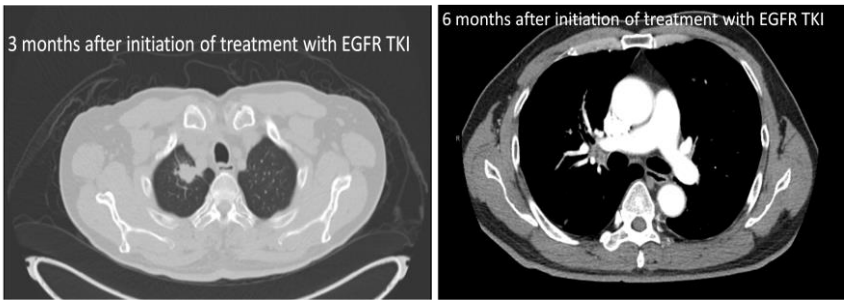
The Case Continues

EGFR status was positive in the patient's lymph node specimens. The risks and benefits of first line chemoradiotherapy versus biomarker-driven therapy were discussed with the patient. Considering his poor lung function, poor performance status, and risk for treatment-related pulmonary toxicity, a shared decision was made to begin treatment with an EGFR TKI.Three months later, a repeat chest CT scan showed decrease in size of the right upper lobe mass and a small interlobar lymph node at station 11R superior, but no mediastinal or other hilar adenopathy. A total of six months after initiation of EGFR TKI therapy, another chest CT scan showed an increase in size of the right upper lobe mass and a small increase in size of the 11R s adenopathy. The patient is discussed again in the multidisciplinary lung cancer conference.



Question 4: What would you recommend next?

A. Switch treatment to conventional chemotherapy
B. Continue current EGFR TKI therapy
C. Re-biopsy the right upper lobe mass
D. TBNA of the right interlobar node

Answer: C

Significant clinical or radiographic response to biomarker-driven therapy is seen initially in many patients with EGFR mutations or ALK rearrangements, but disease commonly progresses as a result of acquired resistance against EGFR and ALK inhibitors.

In patients with documented prior actionable genetic alterations in whom disease has progressed during therapy, retesting should be done exclusively on re-biopsy specimens of a progressing lesion; specifically the lesion that had become clinically relevant. Clinicians should communicate this information with their pathologist to assure that requests for testing on patients with prior history of biopsy are made only for the most recently obtained specimen.

This patient's imaging studies reveal probable disease progression. The benefits of potentially improved outcomes by re-biopsy should be balanced against the risks associated with the use of invasive procedures for tissue acquisition. The location of the tumor and the level of risk involved in the procedure are relevant to deciding whether repeat biopsy is recommended.

For this patient, progression of disease was likely in both the right interlobar node and the primary tumor, but is most obvious in the primary tumor. The primary lesion is therefore the preferred site for rebiopsy.

About 50% of resistant tumors show the T790M mutation, 5% show MET overexpression and PIK3CA mutations. Transformation into small cell lung cancer occurs in about 15%. Targeted treatments against T790M mutations, MET- and PIK3CA are currently available in clinical trials.

While rebiopsy may allow detection of resistance mechanisms and alter therapy, indications for rebiopsy depend on treatment setting and availability of clinical trials and other cancer-related research.

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The Case Continues

The patient was referred to thoracic surgery for possible surgical biopsy to obtain adequate specimens for molecular testing. Based on the patient's tumor anatomy and given the high probability of significant loss of lung function, a surgical resection was not recommended for molecular testing alone in this patient. The patient was re-discussed at the multidisciplinary lung cancer conference.

International guidelines recommend a multidisciplinary approach to patients with limited stage and advanced stage lung cancer

•**British Thoracic Society** Guidelines on the selection of patients with lung cancer for surgery. Thorax 2001; 56:89-108.

•**European Society for Medical Oncology** Clinical Practice Guidelines for diagnosis, treatment and follow-up (Metastatic non-small-cell lung cancer working group); Ann Oncol. 20 12;23 Suppl 7:vii56-64).

Question 5: To optimize the specimen submitted for molecular testing, which of the following items should you discuss with your pathology and oncology colleagues prior to further interventions?

A. Size and quality of the required tumor sample
B. Specific histologic sampling for molecular analysis testing
C. Specific markers needed in this patient
D. All of the above

Answer: D

Attention to the quantity, quality and processing of tissues helps optimize molecular testing. Tumor cellularity appears to be the most significant factor for test success regardless of whether a cytology or pathology specimen is used. The presence of non-malignant tissue within specimens can lead to decreased accuracy of molecular testing, especially when testing is based on nucleic acids extracted from the entire sample.

Molecular testing is currently indicated in non-small cell lung carcinoma showing an adenocarcinoma component regardless of histologic grade or subtype, in small biopsies or incomplete excision specimens showing only squamous or small cell histology, and in poorly differentiated tumors or tumors that otherwise cannot be classified as pure squamous, pure small cell, or pure neuroendocrine carcinomas. Given the emergence of new molecular markers for other histologic types, need for specific tests should be clarified with the referring oncologist prior to tissue acquisition.

Each molecular laboratory has a minimal amount and concentration of tumor cells required for accurate detection of molecular alterations based on specific tumor enrichment protocols available and assay platforms used for testing. The treating team and the pathologist should be aware of these requirements so that the number of specimens rejected by the molecular laboratory is minimized.

References

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The Case Continues

At the lung cancer conference, it was suggested that rebiopsy be performed. Both the oncologist and pathologist suggested that samples for histology and cytology be obtained.

Question 6: Which procedure would you recommend?

A. CT-guided FNA and biopsy of the right upper lobe mass
B. Bronchoscopy with transbronchial biopsy, brushings and lavage from the right upper lobe mass
C. EBUS-guided TBNA from the right interlobar node
D. EBUS –TBNA from the right interlobar node and transbronchial biopsy, brushings, and lavage from the right upper lobe mass.

Answer: D

Empiric evidence suggests patient management is optimized by the use of multidisciplinary lung cancer conferences. Interaction among key specialists avoids a system of serial and autonomous referrals that may delay and fracture care. The active involvement of key specialists, including those most knowledgeable of the optimal use of small specimens for molecular analysis is increasingly relevant. The impact of compliance or discordance with decisions and recommendations made at multidisciplinary conferences warrants further study.

At this conference, pathologists noted that tissue requirements for molecular analysis may exceed those for diagnostic cytologic or histologic examination. The pulmonologist noted that in peripheral parenchymal lesions, a systematic review showed an overall sensitivity of 69% for conventional bronchoscopy with transbronchial biopsy, brushings and lavage, but without electromagnetic navigation or radial ultrasound assistance. Brushing alone had a sensitivity of 52 %, whereas transbronchial biopsy had a sensitivity of only 46%. BAL and washing had a sensitivity of 43%. The size of the lesion impacts yield. The sensitivity of all modalities for peripheral lesions less than 2 cm was 0.33 compared to 0.62 for lesions greater than 2 cm. At least 4-5 biopsies should probably be performed to obtain sufficient material for molecular testing.

The Interventional radiologist said that diagnostic yield for transthoracic CT-guided biopsy and needle aspiration is reportedly greater than 90%. She argued that CT-guided biopsy was the optimal procedure in this patient because adequate quantity and quality of lung tissue can be obtained for molecular testing if multiple passes are performed. Complications include hemorrhage and pneumothorax, which, in high-risk patients might prompt a preference for a bronchoscopic approach. The pulmonologist responded that EBUS-TBNA from the interlobar node could be performed at the time of bronchoscopy with transbronchial biopsy, brushings and lavage from the right upper lobe mass. Using EBUS-TBNA, cytology and tissue cores for histology are obtained that are adequate for molecular testing.

The rates of successful molecular testing with small volume biopsies and cytology specimens obtained by transthoracic and bronchoscopy needle aspiration-biopsy probably vary depending on the *fixation method* and the *extent of analysis* performed. In one study, molecular analysis was successful in only 48% of fresh specimens submitted for gene expression profiling. On the other hand, fresh frozen biopsies from TTNA submitted for EGFR testing alone was successful in 100% of cases.

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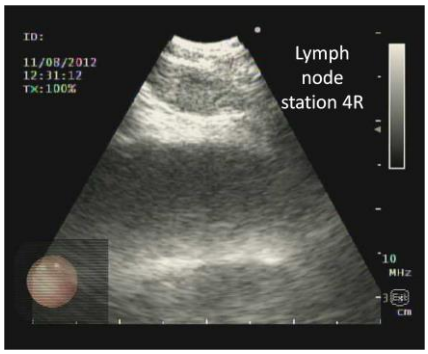
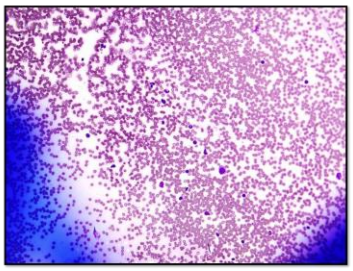
The Case Continues

The multidisciplinary team chose to proceed with bronchoscopy and EBUS-TBNA. White light bronchoscopy showed normal airways. EBUS exploration of the mediastinum showed lymph nodes at stations 4R and 11R superior. EBUS-TBNA from both nodes was attempted. The nodes were very difficult to penetrate. A different inter-cartilaginous space was chosen for puncturing the nodes by redirecting the needle; the 4R lymph node could not be penetrated. Two aspirates of the 11R superior lymph node were obtained. On ROSE, however, only blood and a few scattered lymphocytes were visualized.



Unsatisfactory nodal aspiration: scanty lymphocytes are present on a background of red blood cells. No malignant cells are seen



<u>Question 7</u>: What would you do next?

A. Abort the EBUS procedure and proceed with transbronchial biopsy, brushings and lavage from the right upper lobe mass
B. Change the puncture site again and reattempt EBUS-TBNA from station 4R and 11Rs
C. Perform conventional TBNA of region 4R using a 19 gauge histology needle
D. Abort bronchoscopy and consult interventional radiology

Answer: A

In this case, technical difficulties were encountered while sampling nodal stations 4R and 11R. ROSE confirmed the lack of representative tissues, prompting a change in strategy. Selecting the correct biopsy site for molecular testing may have implications on patient morbidity. When it is difficult to obtain enough tissue because of technical problems or intra-procedural complications, it is important to prioritize acquisition and use of tissue based on which information can be obtained with the limited specimen.

Performing TBNA at all cost by changing the puncture site again, prolonging the procedure, or switching to conventional TBNA may not be in the patient's best interest for at least two reasons: tissue can be obtained from another site of likely disease progression; in this case the enlarging right upper lobe mass, and multiple repeated needle aspirations are likely to result in a bloodier specimen that will be difficult to analyze.

The safety of obtaining a biopsy must be weighed against the need to select biomarker-driven therapy in fragile patients with lung cancer who often have significant cardiac and pulmonary comorbidities.

References

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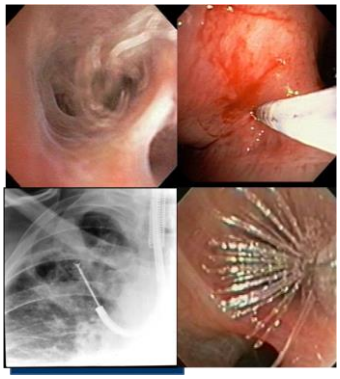
The Case Continues

A conventional TBNA was not performed. The EBUS scope was removed and transbronchial biopsy, brushings and BAL were performed using fluoroscopic guidance.



Question 8: Had TBNA been successful, what should be the major focus of specimen handling in the procedure suite?

A. Prepare as many slides as possible for ROSE
B. Prepare 1-3 slides for histologic diagnosis and save the rest for cell block
C. Perform aspirations until a tissue core is obtained

Answer: B

Slides can be prepared and preliminarily interpreted by cytotechnologists to assure an adequate specimen for molecular testing. This may reduce time, effort, and complications. Physicians and their staff should be familiar with the process of smear preparation. Cytologic sampling is limited by the amount of tissue obtained because a small number of passes are made through the lesion. Often, all the aspirated material is expressed onto a single or small number of slides. Any type of cytologic sample and preparation may be used, including stained or unstained smears, automated Thin Prep slides and cytospin. Cell-block is commonly used for molecular analysis. The proportion of unsatisfactory cytology specimens for molecular analyses varies significantly with the type of cytological specimen. Obtaining core tissue should be attempted but is not always successful.

When cell blocks are planned, only a few slides might be needed for Diff-Quick and Papanicolau smear. The rest of the specimen is saved for cell-block preparation. Material obtained from additional sampling of the lesion or lymph node is expulsed from the needle into a solution such as Cytolyt. This is rendered into a pellet which is embedded in paraffin. This preparation may not represent the underlying tissue architecture, but is adequate for special stains and molecular analysis. Because there is some debate whether alcohol fixation is appropriate for FISH testing, techniques should be discussed with the institution's pathologist.

EBUS-TBNA and pleural fluid are more suitable than BAL because a larger number of tumor cells are usually retrieved, and there is greater possibility to make a cell-block.

Molecular testing for EGFR, K-RAS, BRAF, ALK and PIK3CA on cytology specimens provides results that are equivalent to those obtained from histology specimens in about 80% of cases.

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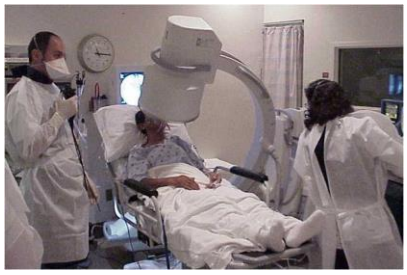
The Case Continues

Using fluoroscopic guidance, two brushings and five transbronchial biopsies were performed, followed by BAL with a total fluid instilled of 120 ml and return of 60 ml of bloody fluid. Results from the biopsy showed adenocarcinoma. The treating oncologist considered reflex molecular testing to obtain a complete molecular profile as soon **a**s possible.



Question 9: At this point, what would you do?

A. Defer molecular testing to the treating oncologist
B. Defer molecular testing to the pathologist
C. Order the tests based on the previous discussions with the oncologist and the pathologist
D. Do not order any tests until after lung cancer tissue acquisition, handling and processing are streamlined at your institution

Answer: C

Reflex testing is also known as automatic testing. It is often done by pathologists at the time of histologic diagnosis to ensure that complete histologic and molecular profiles of the tumor are available to the oncologist as early in the treatment algorithm as possible.

There are reports of discordance in *molecular* alterations between the primary tumor and nodal metastasis with similar histology. In addition, *histologic* discrepancy between the primary tumor and associated lymph nodes with similar molecular alterations has also been reported. Discussions with the referring oncologists and the pathologists are important prior to ordering molecular tests. Streamlining the institution's workflow for molecular markers is desirable.

Experts propose developing an institutional multidisciplinary lung cancer tissue management committee to decide which patients should undergo molecular testing and which tests should be performed. Guidelines from The European Society of Medical Oncologists as well as CAP/IASLC/AMP the guideline do not currently recommend routine testing for KRAS, BRAF, ERBB2, PIK3CA somatic gene mutations outside of clinical trials. Routine testing for mRNA levels of ERCC1, RRM1, TS and BRCA1 is not currently recommended outside of clinical trials.

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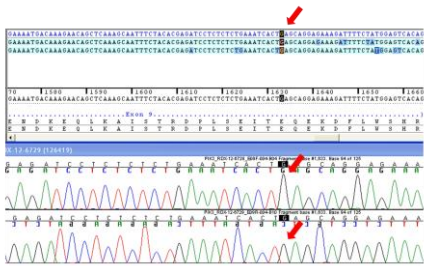
The Case Continues

Reflex testing in this institution applied only to EGFR and KRAS mutations and ALK rearrangement. In this patient, additional studies were desired by the oncologist. The tumor was negative for KRAS and for secondary EGFR mutations, and for PIK3CA mutation but showed c-MET amplification. No ALK rearrangement was noted.

Because currently used molecular markers may be requested even though their impact on clinical outcomes is unclear, the issue of standardizing the molecular testing process was discussed in the multidisciplinary lung cancer conference. The team created a form to accompany all lung cancer patients to their appointments with oncologists, surgeons, interventional radiologists and pulmonologists. The molecular markers included on this form were debated. Other elements included on the form were whether cytology or histology specimens were obtained, the quality of the specimen, and the quantity of adequate specimen for testing. The team decided to individualize the choice of referral molecular laboratories.

Wild type PIK3 by sequencing



Courtesy Response Genetics

Question 10: What elements are critical to help assure that sufficient amount of representative small samples can be processed for molecular analysis?

A. Minimize the number of immunostains for cytohistologic diagnosis
B. Use ROSE whenever possible for FNA procedures
C. Try to obtain tissue cores for histology, obtain specimens for cell block, and document collection time as well as time to fixation.
D. All of the above

Answer: D

Results of molecular tests can alter therapy. In this case, the patient could be enrolled in a clinical trial using a monoclonal antibody against c-MET. An immunohistochemistry panel can aid in sub-classifying NSCLC. Multiple IHC stains, however, may reduce the quantity of tissue available for molecular analysis. Tissue conservation is crucial so that tissue is available for potential molecular testing.

Pathologists should minimize the number of IHC preparations used to sub-classify a lesion. When morphology is equivocal and there is no clear glandular or squamous differentiation, IHC using a panel of antibodies; typically TTF-1, p63 and p40, cytokeratin 7 and cytokeratin 5/6 may be used on previously stained smears, automated slides, and cell-blocks.

Rapid On-Site Evaluation (ROSE), while not available everywhere, is an important complement to needle aspiration. ROSE helps improve the accuracy of the procedure, but does not necessarily improve yield. By using ROSE, the cytopathologist can confirm that the specimen is adequate and representative of the targeted lesion. For example, a significant number of lymphocytes should be found when a lymph node is targeted. ROSE also helps confirm that sufficient material is obtained for a definitive final diagnosis *and* molecular testing. ROSE thus optimizes future allocation and processing of specimens for specific analyses according to suspected or known diagnosis.

Results of IHC usually favor a single diagnosis. Adenocarcinomas of pulmonary origin are typically reactive for TTF-1 and cytokeratin 7 and non-reactive for p63 and cytokeratin 5/6. Squamous cell carcinomas are typically reactive for p63 and cytokeratin 5/6 but non-reactive for TTF-1 and cytokeratin 7. The concordance between needle aspirate and biopsy in NSCLC subtyping is very high (96%), especially when analysis of material in cell-blocks can be performed.

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Credits

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