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Clinical Stem 2

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

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.

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 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.

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.

References:


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.

References

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**

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.

**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.
References:


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 EGFGR, 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.

References:

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:


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:


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:
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


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.
**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.

**References:**


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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.

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