



The importance of a satisfactory biopsy for the diagnosis of lung cancer in the era of personalized treatment

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ABSTRACT

Advances in molecular biology are improving the understanding of lung cancer and changing the approach to treatment. A satisfactory biopsy that allows for histologic characterization and mutation analysis is becoming increasingly important. Most patients with lung cancer are diagnosed at an advanced stage, and diagnosis is often based on a small biopsy or cytology specimen. Here, we review the techniques available for making a diagnosis of lung cancer, including bronchoscopy, ultrasound-guided bronchoscopy, mediastinoscopy, transthoracic needle aspiration, thoracentesis, and medical thoracoscopy. We also discuss the indications, complications, and tissue yields of those techniques, especially as they pertain to testing for molecular markers.

KEY WORDS

Lung cancer, diagnosis, bronchoscopy, endobronchial ultrasonography, thoracoscopy, transthoracic needle aspiration, molecular markers, *EGFR*

1. INTRODUCTION

Discoveries in molecular biology are changing the approach to the treatment of patients with non-small-cell lung cancer (NSCLC). In recent years, the histologic characterization of lung cancer has markedly advanced, moving beyond the simple distinction of small-cell or non-small-cell disease.

The observation that certain histologic subtypes respond differently to particular chemotherapeutic agents and the increasing use of targeted therapies have created a need for precise histologic characterization of biopsy specimens. For example, several trials have shown that response rate and survival with pemetrexed are significantly better in patients with non-squamous histology¹⁻³. Trials using tyrosine kinase inhibitors have observed that greater benefit from treatment with those agents is seen in patients

with NSCLC tumours harbouring *EGFR* mutations than in patients with wild-type tumours⁴. Ongoing clinical trials are examining the use of Alk inhibitors in patients with NSCLC characterized by *EML4-ALK* gene translocations⁵. Those mutations are found almost exclusively in adenocarcinomas.

Lung cancer remains the leading cause of cancer death in North America. In Canada in 2010, an estimated 25,300 Canadians were diagnosed with lung cancer, and 20,600 died of the disease⁶. At diagnosis, 75% of patients have either locally advanced or metastatic disease⁷. The goal in this latter group of patients is to establish the diagnosis and, ideally, to confirm the disease stage with the least invasive technique possible. As a result, biopsy specimens have become increasingly smaller. Of NSCLC patients receiving chemotherapy for advanced disease, 80% will have only a small biopsy specimen or cytology samples available for diagnosis⁸.

In this review, we discuss the minimally invasive and invasive techniques available for the diagnosis and staging of lung cancer, with their success rates and complications. We also discuss the size of the tissue samples obtained by the various techniques, as that size pertains to maximizing the histologic characterization of lung cancer in an era of personalized medicine. Lastly, we recount our own experience in obtaining adequate tissue samples in our Rapid Investigation Clinic at McGill University.

2. DIAGNOSTIC TECHNIQUES

2.1 Fiberoptic Bronchoscopy

The primary diagnostic tool in lung cancer is the bronchoscope. Flexible bronchoscopy, usually performed under local anesthesia and with minimal sedation, provides a thorough examination of all segmental bronchi within minutes. Complications are rare. Surveys of more than 75,000 procedures reveal mortality rates between 0.01% and 0.5%, and major complication rates between 0.08% and 5%⁹.

Complications include pneumothorax, hypoxemia, and hemorrhage¹⁰.

Endobronchial tumour may manifest as submucosal infiltration or an exophytic mass (Figure 1). Forceps biopsies of endobronchial tumour and mucosal abnormalities are performed under direct vision. The diagnostic yield increases when computed tomography (CT) images are available for review before the bronchoscopy, because the bronchoscopist is then better able to localize the bronchial segment containing tumour¹¹.

If the lesion in the bronchus is seen, the diagnostic yield for endobronchial biopsy is 70%–90%¹². Historically, 4 specimens have been shown to be adequate for optimal diagnostic yield in central lesions^{13,14}. When the tumour is located on the lateral wall of the airway, biopsy specimens are difficult to obtain using standard forceps. To optimize specimens, use of a spear forceps that has a small

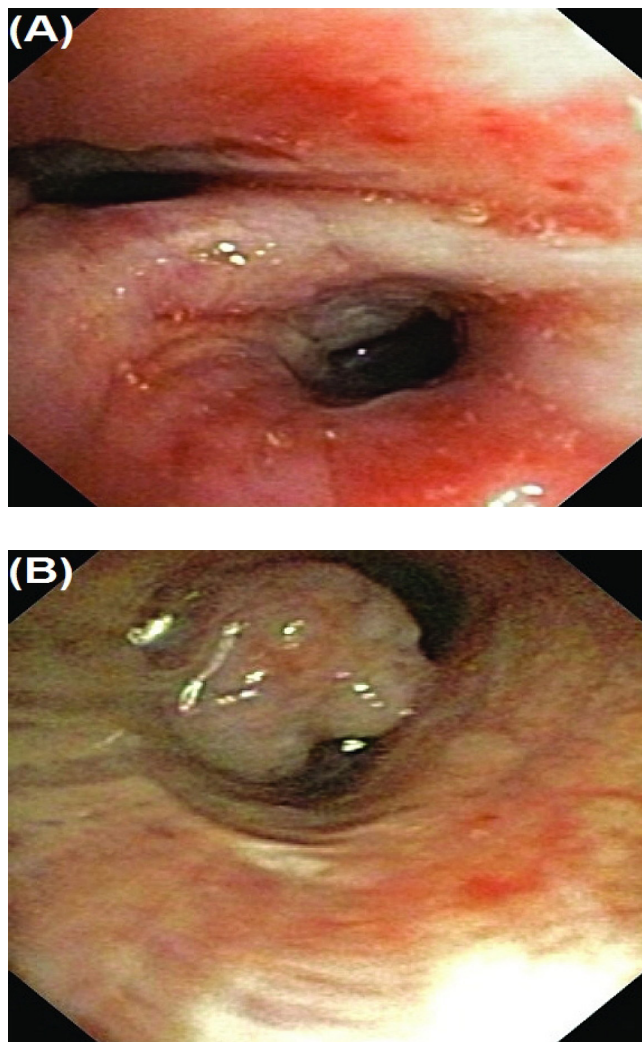


FIGURE 1 Endobronchial tumour. (A) Submucosal infiltration. (B) Exophytic mass.

needle between the biopsy jaws to anchor into the airway wall is recommended. The optimal sequence of techniques for sampling endobronchial disease has been examined by Chaudhary and colleagues. Their study showed that more malignant cells are obtained when bronchial wash is performed after bronchial brushing and bronchial biopsy¹⁵. Most bronchoscopists perform bronchial brushing, biopsy, and washing in that order.

Biopsy specimens are, in general, small—averaging about 300 malignant cells in aggregate biopsies. The amount of tumour contained in the specimens is relatively low. Coghlin *et al.*¹⁶ found that the mean percentage area of tumour in an endobronchial biopsy sample is 33%. In addition, not every biopsy contains tumour. In fact, fewer than half the cases (48%) in the study contained tumour in all biopsy specimens. Although 4 specimens may be enough to make a diagnosis of lung cancer, they may not provide enough tissue to perform a more detailed molecular analysis. Consideration should be given to obtaining up to 6 specimens.

Transbronchial needle aspiration (TBNA) guided by CT images has an established role in the sampling of central adenopathy and masses. However, the technique is underused because of limited training of physicians, fear of complications, and need for on-site evaluation by a cytopathologist to optimize the yield¹⁷. Where the technology is available, “blind” TBNA is increasingly likely to be replaced by TBNA performed under real-time endobronchial ultrasonography (EBUS) guidance.

In the case of peripheral nodules, where the endobronchial exam is normal, the diagnostic yield falls to 40%^{18,19}. When positive, the diagnoses in these cases are usually cytologic, relying on bronchoalveolar lavage or bronchial brushings to make the diagnosis. Ultrasonography techniques using a radial EBUS probe allow for transbronchial biopsy specimens of peripheral nodules to be obtained; this technique is further discussed in the next subsection.

2.2 EBUS Using a Guide Sheath

Endobronchial ultrasonography using a guide sheath and electromagnetic navigation can increase the diagnostic yield of flexible bronchoscopy, especially in the setting of peripheral lung nodules. An increase to 58%–80% from 36% in lesions less than 2 cm in size is possible²⁰. The technique allows for direct visualization of the lesion before biopsy (Figure 2). Advantages of EBUS with a guide sheath are the abilities to analyze the internal structure of lesions, to repeat the access to the bronchial lesion for sampling, and to protect against bleeding from the biopsy site into the proximal bronchus²¹.

Biopsies can be performed using fluoroscopy. In the case of electromagnetic navigation, biopsies are directed in real time to the site of the lesion using a

three-dimensional reconstruction of the airways from CT scans, a steerable probe with position sensor, and an electromagnetic board²².

2.3 EBUS Transbronchial Needle Aspiration

Accurate staging is an essential step in the management of lung cancer patients. The involvement of mediastinal lymph nodes is one of the most adverse prognostic factors in NSCLC because metastases to mediastinal nodes suggest inoperability in most cases²³. Mediastinal staging requires invasive procedures to achieve the necessary tissue confirmation, and mediastinoscopy remains the standard of practice. Transbronchial needle aspiration under EBUS guidance is a novel technique that can achieve minimally invasive staging of the mediastinum. The technique is performed using a dedicated flexible bronchoscope with an integrated ultrasound transducer (Figure 3). It allows for sampling of the mediastinal and hilar lymph nodes under direct vision and is performed as an outpatient procedure in the endoscopy suite, using local anesthesia and moderate sedation. The upper and lower paratracheal, prevascular, and retracheal, and the subcarinal and hilar lymph node stations can all be sampled using this technique²⁴ (Figures 4 and 5).

Studies performed to date indicate that EBUS-TBNA is a safe and accurate method for the mediastinal staging of lung cancer. A randomized trial of conventional

compared with EBUS-guided TBNA demonstrated significantly better yields for EBUS-TBNA in all stations except for the subcarinal²⁵. In 105 patients with suspected lung cancer and enlarged mediastinal nodes, EBUS-TBNA had a sensitivity of 95% and a specificity of 100%. Findings from EBUS-TBNA had a considerable impact on patient management, avoiding 29 mediastinoscopies, 4 video-assisted thoracoscopic surgeries, 8 thoracotomies, and 9 CT-guided lung biopsies²⁶. In the largest study to date, Herth *et al.*²⁷ performed EBUS-TBNA in 502 patients with suspected lung cancer and enlarged mediastinal nodes, comparing the biopsy results with operative findings. The reported sensitivity was 94% and the specificity, 100%. No complications occurred. A meta-analysis of EBUS-TBNA for mediastinal staging in patients with lung cancer reported a pooled specificity of 1.00 (95% confidence interval: 0.96 to 1.00) and a pooled sensitivity of 0.88 (95% confidence interval: 0.79 to 0.94)²⁸.

Endobronchial ultrasound is expected to have an increasingly important role in the diagnosis and staging of lung cancer²⁹. Current guidelines on lung cancer staging recommend that a negative EBUS-TBNA result should be confirmed by mediastinoscopy³⁰. Recent studies have directly compared the diagnostic accuracy of EBUS-TBNA with mediastinoscopy, reporting comparable results for mediastinal staging³¹.

It is important to recognize that there is a learning curve to EBUS-TBNA, with guidelines recommending initial training consisting of 40–50 supervised

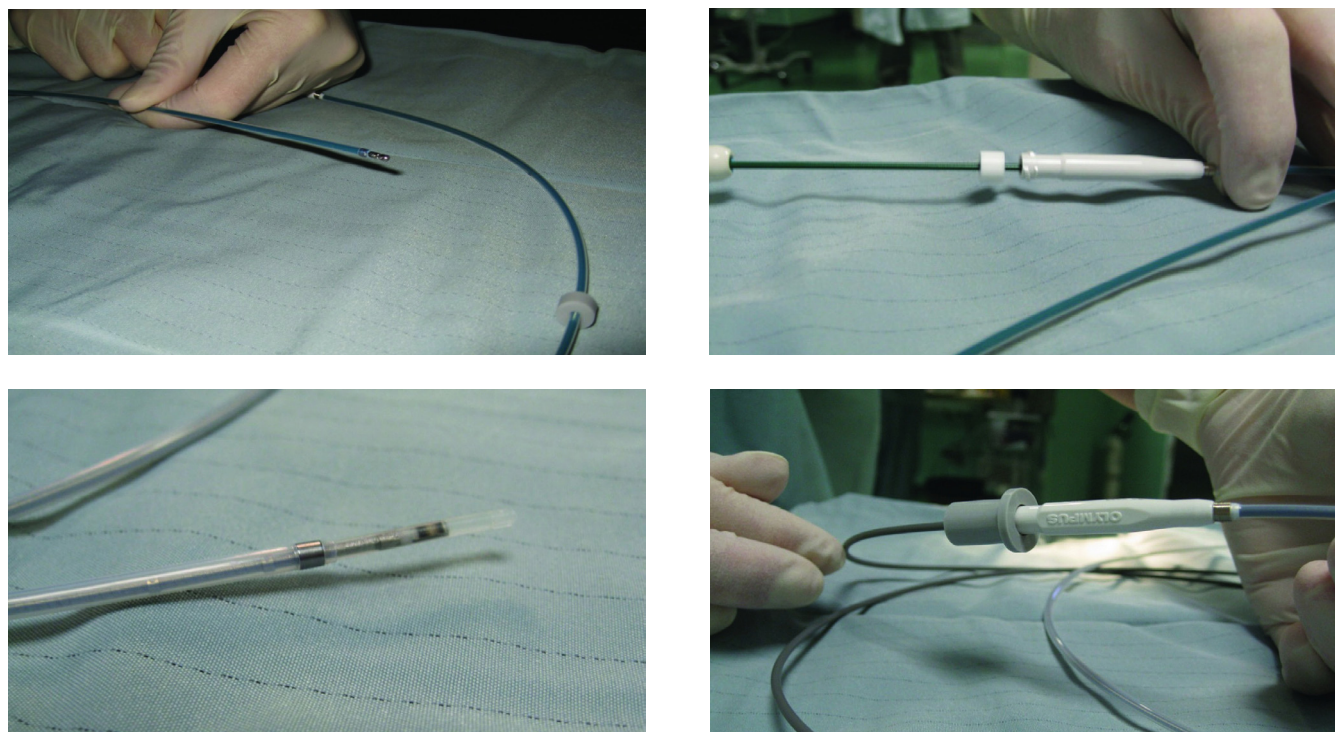


FIGURE 2 Clockwise from top left: biopsy forceps; stopper that marks the biopsy forceps at the correct length; ultrasonography probe; ultrasonography probe and sheath secured with stopper.

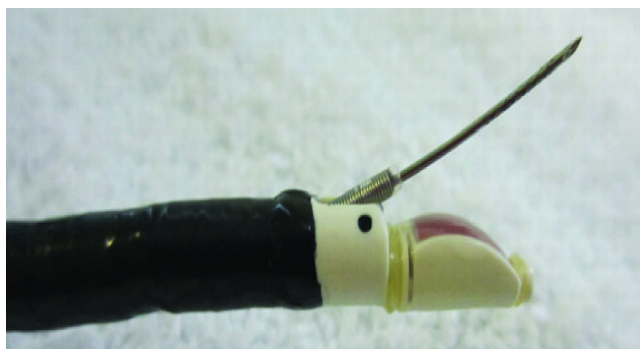


FIGURE 3 Endobronchial ultrasonography needle.

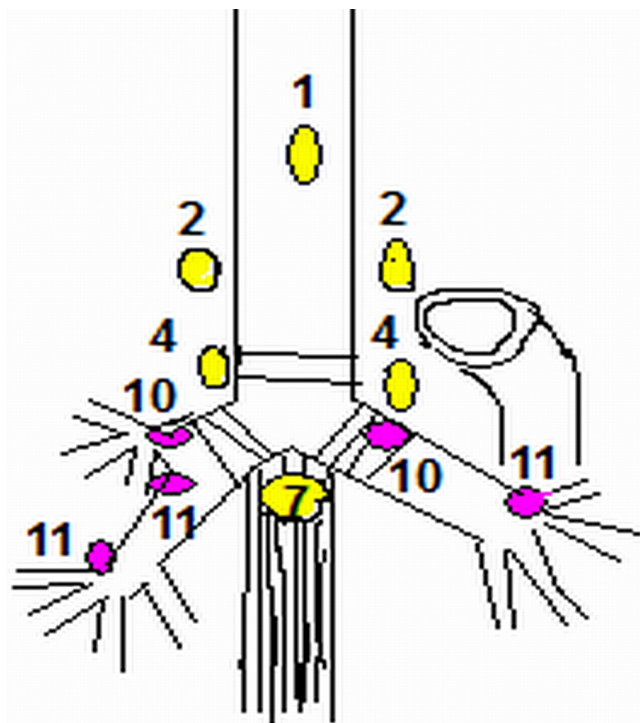


FIGURE 4 Map of mediastinal lymph nodes. (1,2,4,7) Accessible by endobronchial ultrasonography–transbronchial needle aspiration (EBUS–TBNA) and mediastinoscopy. (10,11) Accessible by EBUS–TBNA.

procedures³². However, once optimal diagnostic results are achieved on EBUS–TBNA, confirmation of results may not be necessary. Also, EBUS–TBNA has been successfully used for restaging of the mediastinum, where repeat mediastinoscopy is typically difficult because of fibrotic changes from the earlier procedure³³. One caveat is that the papers published to date come from a small group of authors with extensive expertise, and further studies by different operators are needed.

Tissue samples from EBUS–TBNA are acquired using a dedicated 22-gauge needle, or the more recently introduced 21-gauge needle (Figure 6). Where available, rapid on-site evaluation by a cytopathologist

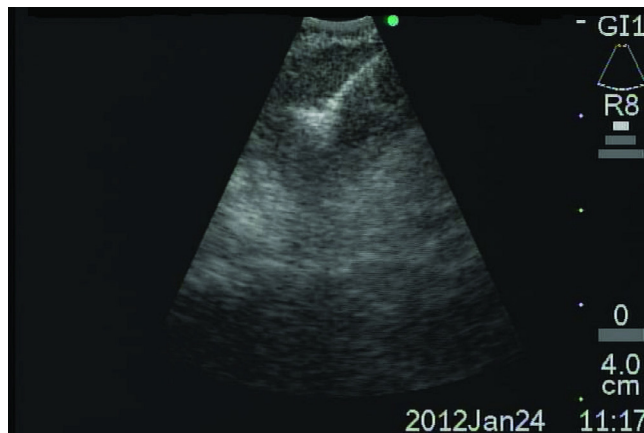


FIGURE 5 Real-time lymph node aspiration.

of aspirated samples is invaluable. In the absence of rapid on-site evaluation, Lee and colleagues³⁴ demonstrated that maximum diagnostic values are achieved with 3 aspirations per lymph node. The needle aspirates are typically processed using a combination of slides and cell block preparations.

Optimal methodology for specimen handling is an important part of improving the yield of EBUS–TBNA, particularly as molecular analysis becomes increasingly important. Nakajima and Yasufuku³⁵ recently reported on their technique to improve the success rate in obtaining histologic cores. The so-called tissue coagulum-clot technique for cell block preparation has been compared with the conventional saline-rinse cell block method, and the former technique was found to significantly increase the cellular yield of cell block preparations³⁶. Several authors have reported on the successful use of EBUS–TBNA specimens for molecular analysis for *EGFR*, *ALK*, and *KRAS* mutations. Nakajima and colleagues reported the successful detection of *EGFR* mutations using DNA extracted from paraffin-embedded EBUS–TBNA samples³⁷. Other groups have reported that molecular analysis for *EGFR* with or without *KRAS* could be performed in 72%–77% of patients with lung adenocarcinoma who had undergone EBUS–TBNA^{38,39}. The results of mutation analysis on cytology aspirates and histologic samples obtained by surgical staging have been compared and found to be reliable⁴⁰.

To summarize: For lung cancer, EBUS–TBNA is an innovative procedure whose main role is in diagnosing and staging patients. Specimens from this technique allow for conventional cytology and molecular mutation analysis. Optimal methodology for specimen handling is key to maximizing the yield of lymph node cytology.

2.4 Mediastinoscopy

Cervical mediastinoscopy is used predominantly in the staging of lung cancer and is considered the

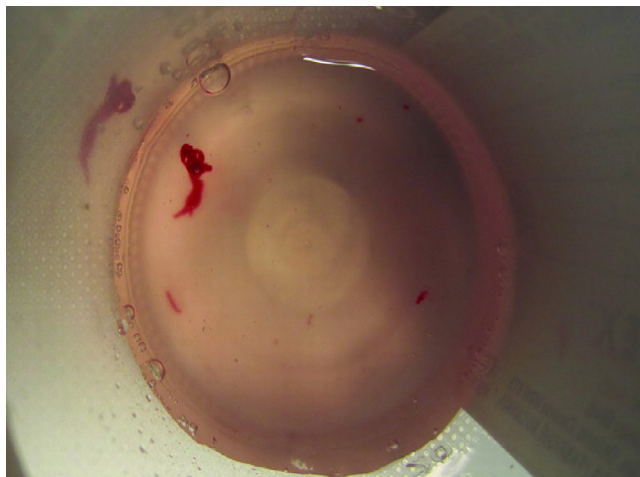


FIGURE 6 Endobronchial ultrasonography needle aspiration sample.

standard of practice in staging the mediastinum. However, EBUS, although still imperfect, is increasingly being used in mediastinal staging. In other words, EBUS is useful to rule nodal metastases in, but not to rule them out.

Cervical mediastinoscopy is performed in an operating room under general anesthesia. A small incision is made at the base of the neck and the mediastinoscope is introduced. The sensitivity of mediastinoscopy for detecting cancer in mediastinal lymph nodes varies between 80% and 95%^{33,41}. False-negative rates of mediastinoscopy vary between 5% and 9% and are attributable to an inability to access the paraesophageal, inferior pulmonary ligament, and aortopulmonary nodes. The tissue samples obtained are on the order of millimetres to centimeters in size depending on the size of the node. Repeat mediastinoscopy cannot easily be performed in the same person because of adhesions and fibrotic changes from the earlier procedure⁴². Complications are on the order of 2%–5% and include hoarseness, infection, and bleeding⁴³.

2.5 Transthoracic Needle Aspiration

In 10%–20% of cases, NSCLC will present as a solitary pulmonary nodule. In patients who are not candidates for surgery or who have advanced disease in which the most accessible site for biopsy is a peripheral lung nodule or mass, transthoracic needle aspiration (TTNA) and biopsy are useful diagnostic procedures.

A TTNA can be performed under either fluoroscopy or CT guidance. Studies comparing the two approaches showed a higher sensitivity for CT guidance⁴⁴. In most centres, a coaxial system, in which a large-gauge needle is inserted into the edge of the lesion and a smaller needle is passed through the larger needle to obtain the specimen, is used. This approach allows for a single pleural puncture, because the larger needle is left in place until all specimens

are collected. The major complications of TTNA are pneumothorax and bleeding. Contraindications to TTNA are previous pneumonectomy; severe chronic obstructive lung disease, especially if large bullae are present; mechanical ventilation; and high risk for bleeding⁴⁵.

The specimens obtained are cutting-needle core biopsies and needle aspirates. Core-needle biopsy specimens usually contain enough cellular material for molecular analysis: approximately 500 cells per core biopsy. Needle aspirates obtained using a 21-gauge needle yield approximately 100 cells⁴⁶. The pooled sensitivity of TTNA for a diagnosis of peripheral bronchogenic cancer is 90% (95% confidence interval: 0.88 to 0.91)⁴⁷. Discordance between TTNA lung cancer cell type and surgical pathology is between 6% and 39%⁴⁷. The false-negative rate with TTNA is relatively high (range: 20%–30%), and so it is not considered a useful diagnostic test to rule out lung cancer¹⁹. If a TTNA is negative, further diagnostic testing is recommended.

2.6 Pleural Fluid Analysis and Medical Thoracoscopy

The choice of technique in the diagnosis of lung cancer depends on the presumed stage of the disease. In patients with suspected lung cancer presenting with an accessible pleural effusion, thoracentesis is recommended to distinguish between a malignant and a paramalignant effusion¹⁹. The yield of pleural cytology is 60%–80% under optimal conditions and repeat sampling^{48,49}. The use of cytology specimens for molecular testing is under evaluation. The 2010 European consensus for *EGFR* testing in NSCLC cautioned that further research was required before testing of cytology specimens could be recommended. Cell blocks of pleural fluid have been used and have a low insufficiency rate of 3.7% (1 in 27 specimens) for *EGFR* and *KRAS* mutation testing⁵⁰.

When 2 cytology specimens are nondiagnostic, or when the material is insufficient for histologic classification, thoracoscopy is recommended. Closed pleural biopsy has a low yield (17% in one series in patients with nondiagnostic cytology)⁵¹. Biopsy performed under direct vision is preferred, because pleural nodules are often scattered on the parietal pleural surface (Figure 7).

Thoroscopic examination of the pleural cavity can be performed using medical thoracoscopy. Unlike surgical thoracoscopy, medical thoracoscopy can be performed in a dedicated sterile endoscopy suite under local anesthesia and conscious sedation. A pneumothorax is artificially induced and a rigid thoracoscope is introduced into the pleural cavity. This procedure has two clinical uses: the diagnosis of pleural effusions, and the application of pleurodesis should an effusion be identified as malignant. Obtained under direct vision, the diagnostic yield of medical thoracoscopy for malignancy is 93%–97%⁵².

Biopsy specimens are about 5 mm in size, and multiple specimens can be obtained.

Medical thoracoscopy is a relatively safe procedure with a major complication rate of 1.9%⁵³. Complications include persistent air leak lasting for more than 7 days, subcutaneous emphysema, and postoperative fever. Mortality is reported to be very rare, with 1 death reported in more than 8000 cases⁴⁹.

3. TISSUE SAMPLING IN THE RAPID INVESTIGATION CLINIC

At the McGill University Health Centre, a “fast-track” diagnostic evaluation clinic was established with the goal of accelerating the outpatient diagnostic assessment of patients with suspected lung cancer. After prompt imaging, preference is given to the invasive diagnostic technique—bronchoscopy, EBUS, TTNA, mediastinoscopy, or open-lung biopsy—that is felt to have the best yield–risk ratio based on CT findings. Procedures that allow for simultaneous diagnosis and staging are favoured.

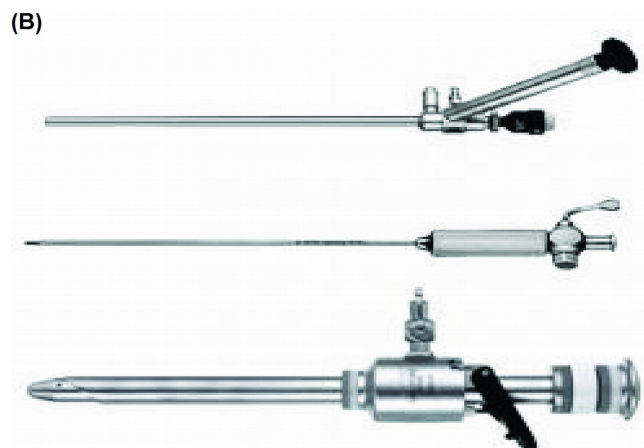
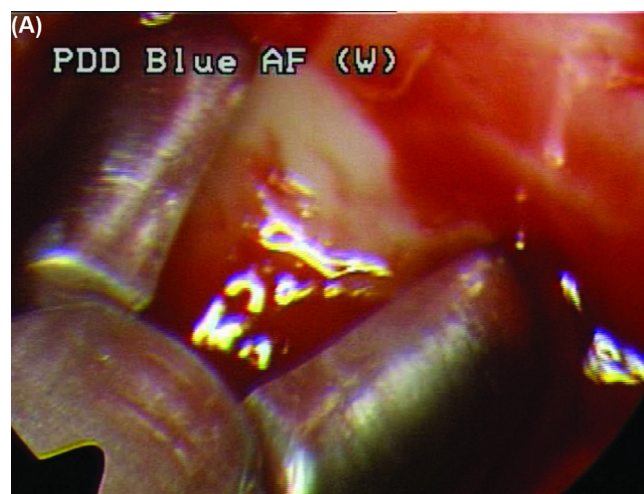


FIGURE 7 (A) Biopsy of pleural nodule. (B) Thorascopes.

Among patients evaluated in the clinic from February 2010 to June 2011, a diagnosis of NSCLC was confirmed in 124: 38 with stage I–II NSCLC and 86 with stage III–IV NSCLC. The first procedure performed to make the diagnosis of lung cancer was flexible bronchoscopy in 36 patients (29%), surgical resection in 10 patients (8%), TTNA in 35 patients (28%), linear EBUS–TBNA in 27 patients (22%), radial EBUS in 7 patients (6%), thoracentesis in 1 patient (0.8%), craniotomy in 1 patient (0.8%), bone biopsy in 1 patient (0.8%), and mediastinoscopy in 2 patients (1.6%). Given their medical comorbidities, 4 patients (3%) were left without pathology confirmation.

Of the 36 patients who initially had a bronchoscopy that confirmed the diagnosis of NSCLC, 18 required further tissue sampling for staging. We reviewed the 18 patients who had no other invasive tissue sampling after bronchoscopy. In 17 of the 18, the pathology specimens consisted of endobronchial specimens varying in size from 1 cm to 0.1 cm. In 1 of the 18, only a bronchial wash was positive by cytology.

Overall, tissue sampling for molecular testing was adequate in 116 of 124 patients. Pathology confirmation of malignancy was not achieved in 4 patients. The specimens that could not be analyzed because of inadequate cellularity included 1 bronchial wash, 1 radial EBUS wash, 1 bone biopsy, and 1 pleural fluid sample.

4. OPTIMIZING SPECIMENS FOR MOLECULAR ANALYSIS

Most routinely available pathology specimens can be used for biomarker analysis, including formalin-fixed, paraffin-embedded tissue and cell block preparations. The minimum number of malignant tumour cells required for biomarker testing is not well established. In general, larger samples with at least 200–400 available malignant cells are preferable⁵⁴.

To avoid overconsumption of tumour for additional immunohistochemistry or biomarker analysis if the clinical and radiographic context is not appropriate, it is important that the pathologist be part of the multidisciplinary team.

Isolation of tumour cells often requires macro- and microdissection. Optimizing testing on extracted DNA means minimizing DNA from non-neoplastic cells in the sample⁸.

5. SUMMARY AND RECOMMENDATIONS

Advances in molecular biology are improving the understanding of lung cancer and changing the approach to treatment. The importance of a satisfactory biopsy that allows for histologic characterization and mutation analysis cannot be overemphasized. In this article, we reviewed the techniques available for diagnosing lung cancer and the tissue yields associated with each technique.

In general, the most easily accessible site should be chosen for biopsy, and a sufficient sample should be obtained. Biopsy specimens are preferable to cytology specimens, but there is increasing evidence to suggest that, when handled judiciously, cytology specimens can be used for molecular analysis. To optimize the analysis of limited tissue available for biomarker testing, it is important that the pathologist be involved in the multidisciplinary team. It is equally important that the respirologist, interventional radiologist, and thoracic surgeon understand the importance of obtaining a satisfactory amount of material, because the specimens obtained ultimately affect the patient's management and prognosis.

6. CONFLICT OF INTEREST DISCLOSURES

The authors have no financial conflicts of interest to disclose.

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