UNIVERSITA' DEGLI STUDI DELL'INSUBRIA

DOTTORATO DI RICERCA IN

CHIRURGIA E BIOTECNOLOGIE CHIRURGICHE

CICLO XXVIII

Coordinatore: Prof. Lorenzo Dominioni

COMPARISON OF MULTIPLE TECHNIQUES FOR ENDOBRONCHIAL ULTRASOUND TRANSBRONCHIAL NEEDLE ASPIRATION (EBUS-TBNA) SPECIMEN PREPARATION IN A SINGLE INSTITUTION EXPERIENCE

Relatore:

Chiar.mo Prof. **Lorenzo Dominioni**

Dottorando:

Dr.ssa **Valentina Conti**

Anno Accademico 2014 – 2015

Introduction

Anatomy of mediastinum

The mediastinum is a thoracic space between the two pleural cavities; it extends from the superior thoracic inlet to the superior surface of diaphragm.

The mediastinum is anatomically subdivided in different compartments, defined as anterior, middle and posterior compartment.

The anterior compartment normally contains the thymus gland, internal mammary vessels, lymph nodes, connective tissue and fat. At times, parathyroid glands and ectopic thyroid tissue may be foudend in the mediastinum.

The middle compartment, called visceral, contains the pericardium, heart and great vessels. The trachea, proximal portions of the right and left mainstem bronchi, and esophagus are the major visceral structures. Lymphatic tissue, the vagus and phrenic nerves, the supra-aortic and para-aortic bodies, multiple nerve plexuses and fibers, the thoracic duct, the proximal portion of the azygos venous system, connettive tissue, and fat are also contained in this compartment [1].

The posterior compartment, contains the proximal portions of the intercostal arteries and veins, proximal portion of the anterior ramus and the rami communincates of the intercostal nerves, thoracic spinal ganglions, sympathetic trunk and connective and lymphatic tissues, as well as the distal azygos vein.

Mediastinal lymph nodes

The mediastium is rich in lymphatics and lymph node aggregates that drain the various organs within the mediastinum, the structures in the neck and portions of those below the diaphragm. These lymph nodes may be the site of localized inflammatory disease, primary lymphatic tumors or metastatic disease from other primary sites.

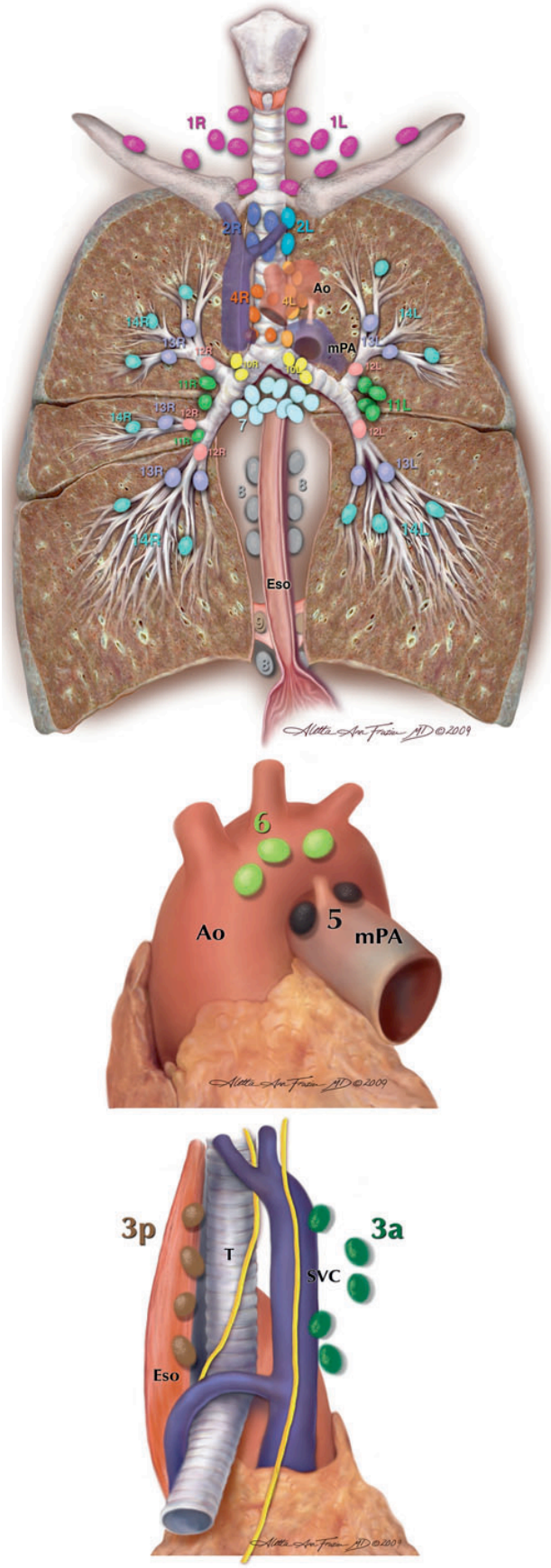
The status of regional lymph nodes is a major factor for staging, assigning treatment, and evaluating treatment efficacy in patients with lung cancer[1].

Lymph node classification

Naruke and colleagues first described a lymph node map of mediastinum and lungs in 1978[2]. In 1997 Mountain and Dresler recommended a classification of regional lymph node stations that

unified the Naruke system and the system advocated by the American Thoracic Society and the North American Lung Cancer Study Group[3]. This map was adopted by the American Join Committee on Cancer (AJCC) and the prognostic factors TNM Committee of the Union Internationale Contre le Cancer at the 1996 annual meeting.

In 2009, the International Association for the Study of Lung Cancer (IASLC) introduced a new lymph node map of the lungs and mediastinum that resulted from an international and multidisciplinary consensus[4]. The latter regional lymph node station map is illustrated in Figure 1.



Supraclavicular zone
1 Low cervical, supraclavicular, and sternal notch nodes

Superior mediastinal nodes

Upper zone
2R Upper paratracheal (right)
2L Upper paratracheal (left)
3a Prevascular
3p Retrotracheal
4R Lower paratracheal (right)
4L Lower paratracheal (left)

Aortic nodes

AP zone
5 Subaortic
6 Para-aortic (ascending aorta or phrenic)

Inferior mediastinal nodes

Subcarinal zone
7 Subcarinal

Lower zone
8 Paraesophageal (below carina)
9 Pulmonary ligament

N1 nodes

Hilar/interlobar zone
10 Hilar
11 Interlobar

Peripheral zone
12 Lobar
13 Segmental
14 Subsegmental

Figure 1 The IASLC lymph node map[4].

Pathological enlargement of mediastinal lymph nodes

Enlargement mediastinal lymph nodes is a common clinical condition. In general 10 mm is considered the upper limit of normal node size. Lymph nodes may be enlarged due to a variety of inflammatory, infectious, or malignant reasons. Hence, it is important to establish a diagnosis and to differentiate between benign and malignant lymph node enlargement.

Mediastinal lymph node enlargement can result from a wide range of pathologies. Node enlargement may be an isolated finding, or it can be associated with other lung pathology. Listed below are conditions than can result in mediastinal lymphadenopathy:

- Primary lung malignancies
- Metastatic malignancies (other primary cancer)
- Sarcoidosis
- Interstitial lung disease
- Mediastinal lymphoma
- Kaposi's sarcoma
- Non lymphomatous pulmonary lymphoid disorders (Castleman disease, lymphomatoid granulomatosis)
- Pulmonary infection

- Occupational lung disease (silicosis, pneumoconiosis)
- Pulmonary manifestation of rheumatoid arthritis

Staging of mediastinal cancer involvement

In the majority of western countries, approximately three-fourths of patients with lung cancer at the time of diagnosis present with locally advanced or metastatic disease. The remaning 20-25% of patients present with clinical diagnosis of early stage lung cancer (Stage I-II), generally resulting from an incidental finding or from screening, and are potentially amenable to radical surgical treatment. With the exception of the advanced stage patients who are not candidate to any active treatment because of comorbidity and/or compromised performance status, accurate staging of lung cancer is a pre-requisite for deciding the appropriate treatment[5]. The algorithms that where developed in recent years to help deciding the best treatment modality for each patient (surgery or chemotherapy or radiotherapy or a combination thereof) are based on the careful assessment of lung cancer histotype and of mediastinal lymph nodes' pathological status[6-8]. Moreover the diagnosis of a number of molecular markers

of lung cancer (EGFR, ALK, KRAS etc) has become widely accepted as a means to guide the administration of targeted therapy.[9,10]

Thus, adequate tissue sampling of mediastinal lymph nodes for cyto/histological assessment and for molecular studies has became of paramount importance for rational planning of lung cancer therapy.

Several techniques are available for sampling mediastinal lymp nodes, and their use depends on the local expertise available. These techniques include *imaging* by chest CT scan and PET-CT scan (figure 2); *endoscopy*, thus [conventional transbronchial needle aspiration (TBNA), endoscopic ultrasonography - esophageal ultrasound needle aspiration (EUS) and endobronchial ultrasound-transbronchial needle aspiration (EBUS-TBNA)]; and surgery [cervical mediastinoscopy, video-assisted thoracoscopic surgery (VATS), video-assisted mediastinoscopic lymphadenectomy (VAMLA) and transcervical extended mediastinal lymphadenectomy (TEMLA)].

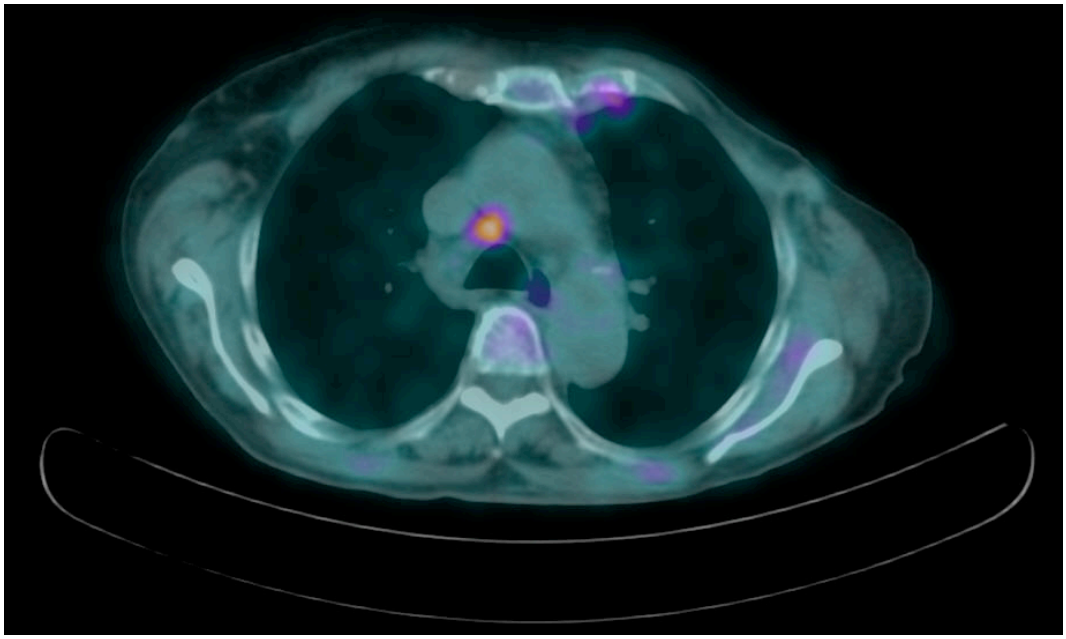


Figure 2. Enlarged mediastinal lymph node (R4 station) visualize as hot spot with PET-CT

Indication of EBUS-TBNA

The most common indications for EBUS-TBNA are: lymph node staging of lung cancer, investigation of mediastinal/hilar masses and mediastinal lymph node enlargement.[11]

Lung Cancer Staging

The current recommendations for invasive mediastinal staging by sampling lymph node stations include all cases of diagnosed lung cancer without distant metastasis, with the exception of cases classified as Stage Ia (clinical/radiological staging) with negative lymph nodes involment at PET/CT. Various methods have been used

for lung cancer staging. Both CT and PET/CT are widely used for this purpose. A prospective study involving 102 patients compared the accuracy of CT, PET/CT, and EBUS-TBNA for the detection of lymph node metastasis from lung cancer. With the exception of patients with extensive N2 disease (bulky disease or involvement of multiple ipsilateral lymph node stations) and N3 disease diagnosed by EBUS-TBNA, the other patients underwent lobectomy with lymph node dissection, making it possible to confirm the EBUS-TBNA cytohistological findings. The diagnostic accuracy of CT, PET/CT, and EBUS-TBNA was 60.6%, 72.5%, and 98.0%, respectively, the differences between the groups being significant ($p < 0.0001$).[12]

A recent prospective study comparing EBUS-TBNA and mediastinoscopy demonstrated that the results of both methods were similar in terms of mediastinal staging in 91% of the evaluated patients. The sensitivity, NPV, and diagnostic accuracy were 81%, 91%, and 93%, respectively, for EBUS-TBNA, compared with 79%, 90%, and 93%, respectively, for mediastinoscopy. There were no statistically significant differences between the two methods.[13]

Lung cancer restaging after neoadjuvant chemotherapy

For lung cancer restaging after neoadjuvant therapy, it is necessary to identify the patients who demonstrate reduction in staging and therefore potentially benefit from surgical treatment.

A prospective study included 61 patients with stage IIIa and IIIb lung cancer as determined by EBUS-TBNA after neoadjuvant chemotherapy. This patients presented with stable lymph nodes or with partial response after chemotherapy as demonstrated by chest CT scans, and there were fit for surgery. All of them underwent restaging by EBUS-TBNA. The patients with cancer-negative EBUS-TBNA results underwent transcervical extended mediastinal lymphadenectomy for histological confirmation. Lymph node metastasis was detected by EBUS-TBNA in 18 (30%) of the 61 patients and in 22 (26%) of the 85 aspirated nodes. Metastatic lymph nodes were identified in 9 (15%) of the 43 patients who underwent transcervical extended mediastinal lymphadenectomy in 7 patients, at lymph node stations accessible by EBUS-TBNA and, in 2 patients, at a station not accessible by EBUS-TBNA. The sensitivity, specificity, accuracy, PPV, and NPV of EBUS-TBNA for detection of lymph node metastasis in restaging were 67%, 86%, 80%, 91% e 78%, respectively.

The authors concluded that EBUS-TBNA is an effective and safe method for mediastinal restaging of patients with non small cell lung cancer.[14]

Mediastinal tumors

Many studies have demonstrated good diagnostic yield of EBUS-TBNA in mediastinal or peribronchial lesions. Yasufuku et al. studied 140 patients with mediastinal tumors in the absence of lung cancer or extrapulmonary cancer. The EBUS-TBNA was diagnostic in 93.6% of all disorders.[15]

Other causes of mediastinal enlargement

The EBUS-TBNA procedure has been used in the investigation of mediastinal and hilar lymph node enlargement in benign and malignant non-pulmonary disease such as lymphoma, sarcoidosis and mediastinal lymp node tubercolosis.

EBUS-TBNA technique

Endobronchial ultrasound guided transbronchial needle aspiration (EBUS-TBNA) adds another minimally invasive tool for the diagnosis

and staging of intrathoracic malignancies. EBUS-TBNA may reach multiple lymph node stations including paratracheal levels (station 2 and 4), subcarinal (station 7) and even hilar nodes (station 10 and 11). Bronchoscopy is performed with a flexible videobronchoscope with a 7.5-MHz linear ultrasound transducer probe (Figure 3).

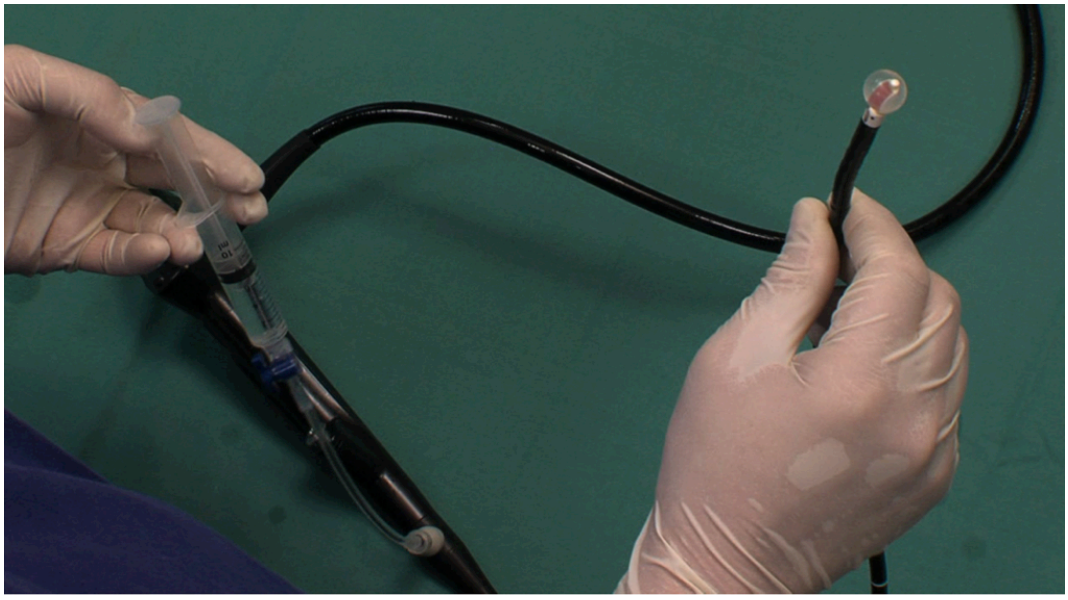


Figure 3 EBUS videobronchoscope with ballon tip.

All accessible lymph node stations are examined and the biopsy is carried out, based on imaging studies, starting with the nodal level that would yield the lowest-stage disease, to avoid contamination. A 22 or 21-gauge needle is placed throught the operative channel to biopsy the targeted node, under realtime ultrasonic guidance (Figure 4 and 5). Doppler examination can be used to prevent unintended pucture of vessels.

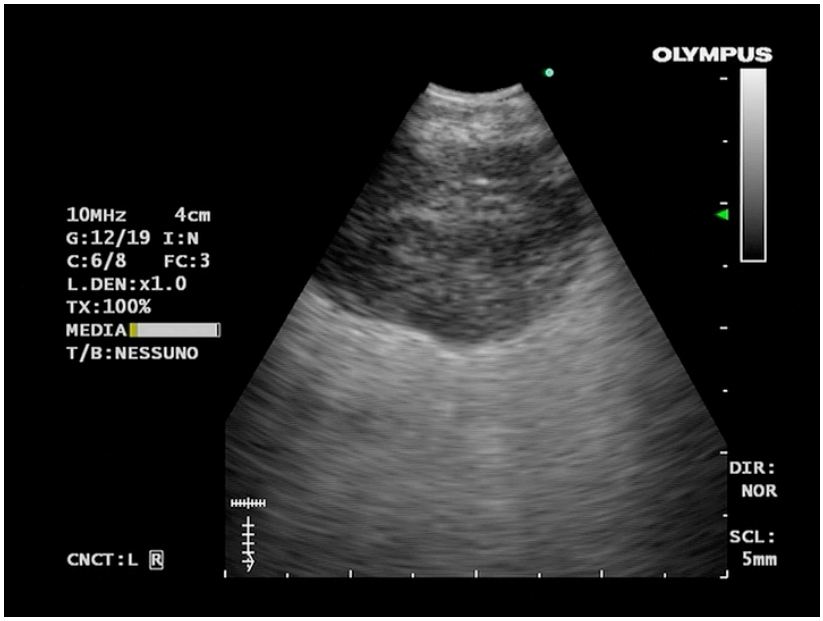


Figure 4 View of mediastinal lymph node in EBUS

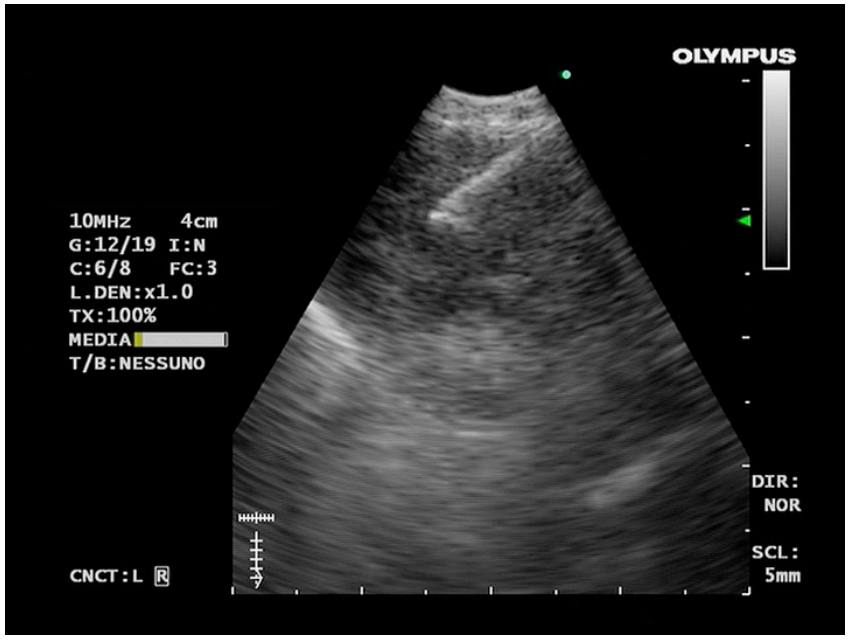


Figure 5 Lymph node biopsy under real time ultrasonic guidance

Limitations, contraindications and complications of EBUS

The limitations of EBUS are: difficult airway examination, due to anatomic anomalies, false-negative results of biopsies, inability to reach certain lymph nodes stations (station 5 and 6), and time and training required to obtain optimal samples.[16]

Contraindications are similar to those of usual flexible bronchoscopy. Absolute contraindications are severe arrhythmia, heart failure, severe hypoxia. Other contraindications related to increased bleeding risk in patients with antiplatelet or anticoagulation treatment, thrombocytopenia and elevated blood levels of urea and creatinine.[17]

EBUS-TBNA is usually a safe procedure; complications being rare.

This complications consist of pneumomediastinum, pneumothorax and hemomediastinum.

Usually, at the end of the procedure, chest X-ray exame is perfomed, but it is not mandatory. Because of the real-time ultrasound imaging, major vessel puncture is theoretically less likely compared with conventional transbronchial needle aspiration (cTBNA). Major vessel puncture (aorta) has been described during cTBNA. Although unintended puncture of major vessels can lead to hematoma, this is

usually clinically non relevant.[18,19] Infectious complications have also been rarely reported.[20]

EBUS-TBNA specimen preparation techniques

Adequate preparation of specimens for cytology is essential for ensuring that EBUS-TBNA has good diagnostic yield. Procurement of adequate specimens is key to provide a specific histologic and molecular diagnosis of lung cancer.

In the literature several techniques for specimen acquisition and preparation have been described, but no comparison of these multiple techniques has been performed. Usually, cytology slides are adequate for the diagnosis of lung cancer and its subclassification, but the material is genarally inadequate for molecular testing. Cell block formation usually improve the ability to determine lung cancer subclassification and allows molecular testing.

Cytology slides were prepared as follows: the sample is placed on the cytology slide, another slide or a coverslip is placed against the first slide at an angle of 45° or is placed horizontally on top of it and is gently slid along the base slide until it reaches the opposite end, causing the liquid sample to spread uniformly. The sample can than

be fixed with 95% ethanol. The slide can be stained 1 minute with heamatoxylin for rapid on-site evaluation (ROSE) by a cytopathologist; later the Papanicolau staining with orange A and eosin can be completed in the laboratory.[21]

Cell block was obtained placing the sample into formaline. Sample is then centrifuged, concentrating the cellular material that was in suspension. The fragments as well as the resulting sediment are embedded in paraffin as a tissue, making it is possible to obtain histological sections that will be examined using histochemistry or immunohistochemistry.[21]

Core-tissue specimens were prepared as usual histologic sample.

Methods

The optimal method for EBUS-TBNA specimen preparation is controversial. Specimen acquisition and preparation are key to obtain adequate tissue for diagnosis, accurate and complete staging of lung cancer with EBUS TBNA. Thus, conventional cytology staining does not always provide sufficient information and more material is often required to plan adequate treatment.

Multiple techniques for specimen acquisition and preparation have been reported in the literature though no direct comparisons of these techniques have been performed until now within the same institution. Rather, the results of different techniques used by different authors were compared[22]; thus different levels of expertise across centers may have influenced the comparison.

In this study we compared multiple techniques for EBUS-TBNA specimen acquisition and processing in a single-institution experience.

We compared five specimen-processing techniques that we used over the study period 2012-2014: 1) cytology slides; 2) cell-block; 3) core-tissue; 4) cytology + cellblock; 5) cytology + core tissue.

This study was approved by the our institutional review board.

We have retrospectively reviewed the data of 199 consecutive patients (71% male; mean age 61±15 SD years) undergoing EBUS-TBNA of mediastinal-hilar lymphadenopathy (lymph node mean size, 27+13 SD mm) for suspect lung cancer metastasis (n=139) or granulomatosis (n=60) at the Center for Thoracic Surgery, Insubria University-Varese, in 2012-2014.

All procedures were performed by two experienced bronchoscopists on rotation, using 21-G or 22-G needle, 3 passes, aspiration, without rapid on-site evaluation (ROSE). Specimens were read by the same expert thoracic pathologists.

EBUS-TBNA procedures

Under a moderate sedation by intravenous fentanyl, midazolam and lidocaine topical anesthesia, standard bronchoscopy was performed for initial inspection and visualization of the airways (Olympus BF180 serie videobronchoscope). After this first evaluation the ultrasound examination (EBUS broncoscope Olympus convex probe 7.5 MHz) was introduced into the airway and ultrasound scanning of the mediastinum and hilum was performed to identify visible lymph nodes. After lymph node identification under realtime ultrasound

guide, the samples were taken with 22 or 21 gauge needle. The stylet inserted into the needle was used to clean bronchial or cartilage fragments and then negative suction was connected by vacuum-lock syringe.

At least 3 specimens were obtained for each lymph node with 15 excursions each time.

The collected material was prepared with different modalities over the study period.

Cytology slide

In first time we prepared always a cytological slide crawled two slides and using both air-dried and wet-fixed methods and then was stained using Papanicolau test.

Cell-block

To prepared cell-block the sampled material was pushed out of the needle with the wire stylet directly into an integrity cellular storage solution to obtain a tissue coagulum clot and then the sample was processed to get 4-5 micron section on glass slide. The sections were used to assess cellularity and morphology.

Core tissue

The sampled core-tissue was prepared pushed out directly in formaline solution and treated as a histological issue.

Cytological slides were read in combination with cell-block or core-tissue for each sample, to assess the impact of dual-modality reading.

To assess the processing-technique diagnostic accuracy in cases with cancer-negative EBUS-TBNA finding, we used as diagnostic gold standard respectively the surgical N status in operated lung cancer patients, or 1-year follow-up.

We evaluated the diagnostic yield, accuracy and area under the curve (AUC) of each of the five mentioned techniques.

Results

In the majority of cases, EBUS-TBNA was performed to obtain the diagnosis of lymph node under examination; only in 3 patients was EBUS-TBNA made for lung cancer staging.

The sampled lymph node stations were: #7 in 60% of patients; #4R/4L in 32%; #10R/11R/10L/11L in 7%; #2R/2L in 1%.

Mean duration of the procedure was 24 ± 9 minutes. No complication occured.

Five different specimen-processing techniques were evaluated and compared over the study period, in sequential order: cytology slides (n=42 patients); cell-block (n=25); core-tissue (n=60); combination of cytology slides+core-tissue (n=51); combination of cytology slides+cell-block (n=21).

Diagnostic yield, accuracy and area under the curve (AUC) were as follows. Cytology slides: 81%, 80%, 0.90; cell-block: 48%, 33%, 0.67; core-tissue: 87%, 99%, 0.96; cytology slides+core-tissue: 81%, 100%, 1.00; cytology slides+cell-block: 86%,100%, 1.00 (Figure 6 and 7). Cytology slides and core-tissue methods showed not significantly different diagnostic yield (p=0.435) and AUC (p=0.152).

We also evaluated the indipendent predictors of diagnostic accurancy by multivariate analysis, shown Table 1. The only independent predictor of diagnostic accuracy for malignancy was the sample processing technique ($p<0.01$).

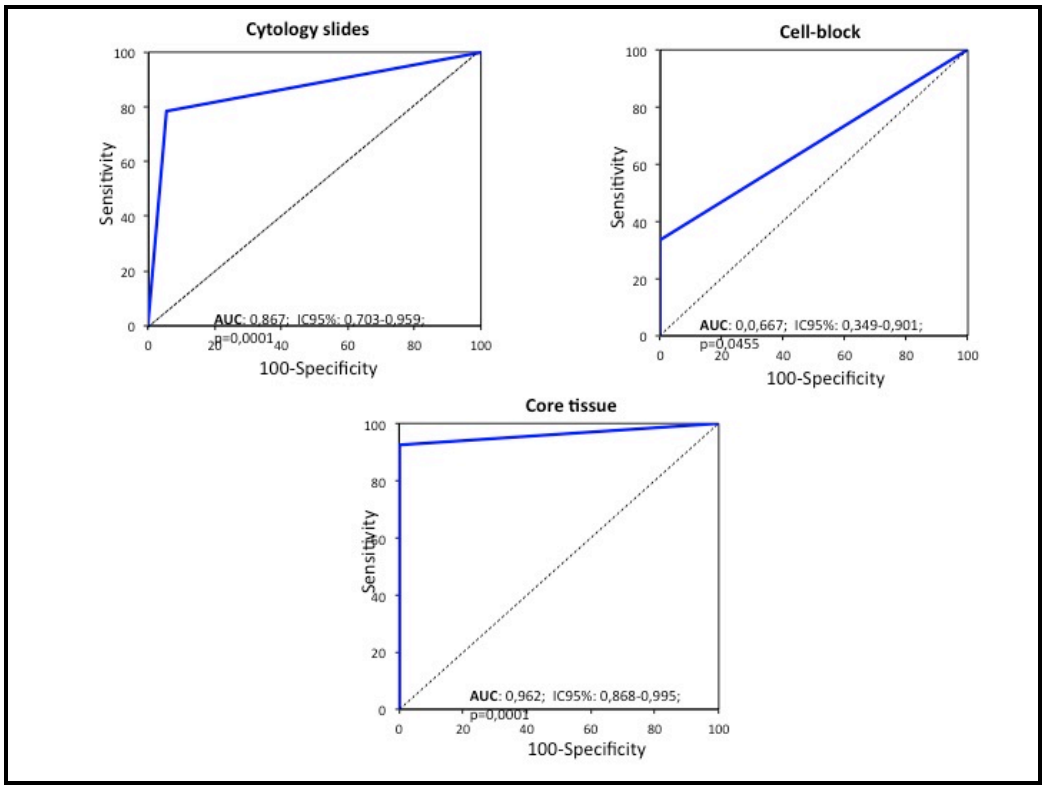


Figure 6 AUC of cytology slides, cell/block and core biopsy

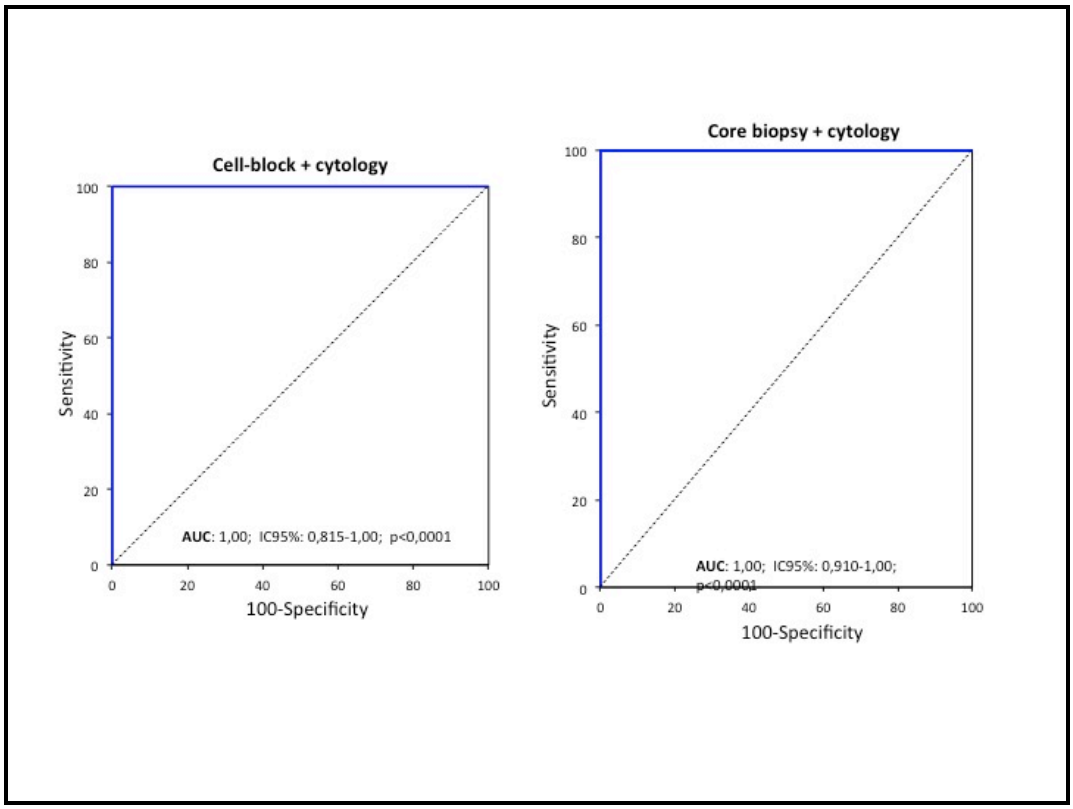


Figure 7 AUC of combination of cytology with cell block and core biopsy

Table 1 Predictors of diagnostic accurancy of EBUS-TBNA

	UNIVARIATE			MULTIVARIATE		
	OR	**95% CI**	***p***	**OR**	**95% CI**	***p***
Progressive number of cases (1-50 v 51-100 v 101-150 v 151-199)	1.14	0.75-1.74	0.52	1.25	0.74-2.11	0.41
LN diameter (> 10 mm)	1.01	0.98-1.04	0.49	1.01	0.98-1.04	0.37
Needle (21G v 22G)	1.52	0.66-3.50	0.32	1.83	0.72-4.62	0.21
Thoracic surgeon v Pulmonologist	1.28	0.64-2.54	0.48	1.26	0.59-2.66	0.55
Specimen preparation	0.62	0.44-0.87	**0.01**	0.54	0.37-0.78	**<0.01**
Disease (malignant v benign)	1.29	0.64-2.61	0.47	1.26	0.74-2.11	0.59

From: Rotolo N et al. Factors affecting accuracy of EBUS-TBNA during the learning curve. To be presented to 19th World Congress for bronchology and interventional pulmonology, Florence, May 2016 (with permission)[23].

Discussion

EBUS-TBNA has become the first-line tool for staging and diagnosis of patients with suspected/diagnosed lung cancer. Adequate specimen acquisition during EBUS-TBNA is very important to achieve a definitive diagnosis and, in patients already diagnosed, to perform mediastinal staging of lung cancer.

In high volume centers, ROSE is used in daily clinical practice. This technique offers an immediate and accurate feedback on the diagnosis and quality of specimen, and the number of needle aspirations may be reduced; however there are no randomized studies supporting the use of ROSE.

Molecular analysis can be routinely performed on the majority of cytological samples obtained by EBUS-guided and conventional TBNA[9,10]. However, the outcome of molecular analysis largely depends on the absolute number of vital tumor cells, percentage of tumor cells present in the material and the sensitivity of the molecular test that is being utilized; both smear and cell block preparations or core tissue can be utilized for molecular testing.[9,10]

Van der Heijden et al in 2014 published guidelines for acquisition and preparation of transbronchial needle aspiration specimens to be used

for the diagnosis and molecular testing of patients with lung cancer.[22] They presented an evidence-based guideline to optimize the tissue sampling procedure outcome in daily clinical practice. There is enough evidence that 3 needle aspirations during EBUS-TBNA provide maximum yield; the needle size (whether 21 or 22 G) does not influence the diagnostic yield. Van der Heijden et al[22], reviewed the pertinent literature, to evaluate if specimen preparation techniques can affect the quantity of biologic material retrieved and accurancy of specimen diagnosis. There are no pubblished trials comparing cell-block and core-tissue techniques; both were found valuable for diagnosis. According to Van der Heijden et al[22] there does not appear to be a superior method for specimen preparation, the optimal specimen preparation depending on the preference and expertise of the local pathologist. However these conclusions were based on comparison of specimen acquisition methods between different centers, and different levels of expertise across centers may have influenced the comparison.

A spanish study[21] evaluated the contribution of EBUS-TBNA with cell block analysis to the diagnostic yield in lung cancer; in this study cell-block improved the pathologic diagnosis attained with conventional

smears (7.7%). Cell block obtained during EBUS-TBNA allowed EGFR mutation analysis in 60% of cases and other clinically relevant information in 30% of those participating in the study.[21]

In the present study, in order to avoid confounding from across-centers comparison, for the first time we compared multiple techniques of EBUS-TBNA specimen acquisition and processing performed in a single-institution experience (Ospedale di Circolo di Varese, Center for Thoracic Surgery and division of Pneumology).

The main result of our study is that cytology slides and core-tissue preparations had high and similar diagnostic performance (Figure 6 and 7). Combination of cytology slides with core-tissue, or with cell-block, showed the highest performance; however it is important to note that combination methods are more expensive and time-consuming.

Conclusion

In conclusion, after comparing different EBUS-TBNA specimen acquisition technique in a single center, and after considering the ongoing discussion in the pertinent literature we think that the best technique to obtain the tissue specimen for a definitive diagnosis depends on the preference and expertise of the pathologist in each center. A multidisciplinary effort and collaboration between EBUS bronchoscopists and cytopathologists is required to achieve the highest diagnostic accuracy of EBUS-TBNA procedures.

References

1. Shields TW, Locicero J, Reed CE, Feins RH. General thoracic surgery – seventh edition. Wolters Kluwer Lippincott Williams & Wilkins. 2009 The Netherlands.
2. Naruke T, Suemasu K, Ishikawa S. Lymph node mapping and curability at various levels of metastasis in resected lung cancer. J Thorac Cardiovasc Surg. 1978;76:832–9.
3. Mountain CF, Dresler CM. Regional lymph node classification for lung cancer staging. Chest 1997;111:1718
4. Rush VW, Asamura H, Watanabe H et al. The IASLC lung cancer staging project: a proposal for a new international lymph node map in the fourthcoming seven edition of TNM classification for lung cancer. J Thorac Oncol 2009;4:568–77
5. De Leyn P et al. ESTS guidelines for preoperative lymph node staging for non-small cell lung cancer. Eur J Cardiothorac Surg 2007;32:1-8.
6. Wei Yung et al. Improvement od cellularity on cell block preparations using the so-called tissue coagulum clot method during endobronchial ultrasound guided transbronchial fine-needle aspiration. Cancer cytopathology 2012;120:185-95.

7. Izumo T et al. Comparison od two 22 G aspiration needle for histologic sampling during endobronchial ultrasound guided transbronchial needle aspiration (EBUS-TBNA). Jpn J Clin Oncol 2014;44:841-5.
8. Vanderlaan PA et al. Endobronchial ultrasound guided transbronchial needle aspiration (EBUS-TBNA): an overview and update for the cytopathologist. Cancer cytopathology 2014;122:561-76.
9. Tennan DA et al. Targeting metabolic transformation for cancer therapy. Nat Rev Cancer 2010;10:267-77.
10. Garcia-Olive I et al. Endobronchial ultrasound guided transbronchial needle aspiration for identifying EGFR mutations. Eur Respir J 2010;35:391-5.
11. Figueiredo VR, Jacomelli M, Rodrigues AJ, Canzian M, Cardoso PFG, Biscegli Jatene P. Current status and clinical applicability of endobronchial ultrasound-guided transbronchial needle aspiration. J Bras Pneumol. 2013;39(2):226-237
12. Yasufuku K, Nakajima T, Motoori K, Sekine Y, Shibuya K, Hiroshima K, et al. Comparison of endobronchial ultrasound,

positron emission tomography, and CT for lymph node staging of lung cancer. Chest. 2006;130(3):710-8

13. Yasufuku K, Pierre A, Darling G, de Perrot M, Waddell T, Johnston M, et al. A prospective controlled trial of endobronchial ultrasound-guided transbronchial needle aspiration compared with mediastinoscopy for mediastinal lymph node staging of lung cancer. J Thorac Cardiovasc Surg. 2011;142(6):1393-400.
14. Szlubowski A, Herth FJ, Soja J, Kołodziej M, Figura J, Cmiel A, et al. Endobronchial ultrasound-guided needle aspiration in non-small-cell lung cancer restaging verified by the transcervical bilateral extended mediastinal lymphadenectomy--a prospective study. Eur J Cardiothorac Surg. 2010;37(5):1180-4
15. Yasufuku K, Nakajima T, Fujiwara T, Yoshino I, Keshavjee S. Utility of endobronchial ultrasound-guided transbronchial needle aspiration in the diagnosis of mediastinal masses of unknown etiology. Ann Thorac Surg. 2011;91(3):831-6.
16. Dincer HE. Linear EBUS in staging non-small cell lung cancerand diagnosing benign disease. J Bronchol Intervent Pulmonol 2013;20:66–76.

17. The British Thoracic Society Bronchoscopy Guideline Committee: a sub-Committee of the Standards of Care Committee of the British Thoracic Society. British Thoracic Society guidelines on diagnostic flexible bronchoscopy. Thorax. 2001;56(suppl 1):11–21.
18. Agli LL, Trisolini R, Burzi M, et al. Mediastinal hematoma following transbronchial needle aspiration. Chest. 2002;122:1106–1107.
19. Kucera RF, Wolfe GK, Perry ME. Hemomediastinum after transbronchial needle aspiration. Chest. 1986; 90:466.
20. Haas AR. Infectious complications from full extension endobronchial ultrasound transbronchial needle aspiration. Eur Respir J. 2009;33:935–938.
21. Sanz-Santos J, Serra P, Andreo F et al. Contribution of cell blocks obtained through endobronchial ultrasound-guided transbronchial needle aspiration to the diagnosis of lung cancer. BMC Cancer 2012;12:34-41.
22. Van der Heijden EHFM et al. Guideline for the acquisition and preparation of conventional and endobronchial ultrasound guided transbronchial needle aspiration speciments for the

diagnosis and molecular testing of patients with known or suspected lung cancer. Respiration 2014;88:500-17.

23. Rotolo N, Crosta G, Nardecchia E et al. Factors affecting accuracy of EBUS-TBNA during the learning curve. To be presented to 19th World Congress for bronchology and interventional pulmonology, Florence, May 2016 (with permission).