Ten-year experience with endobronchial ultrasound-guided transbronchial needle aspiration: single center results in mediastinal diagnostic and staging

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Background: Endobronchial ultrasonography with transbronchial needle aspiration (EBUS-TBNA) is recognized as an accurate and minimal invasive procedure for diagnosis and staging of lung cancer and lymph nodal malignancies. EBUS is recommended as the first choice procedure for mediastinal staging in lung cancer in international guidelines.

Methods: A retrospective evaluation was performed on single center experience with EBUS-TBNA in our thoracic surgery department in a 10-year time frame. Main indication for the procedure was suspected non-lymphomatous malignancy in intrathoracic lymph-nodes on computed tomography (CT) or positron emission tomography (PET) scan. All procedures were performed under conscious sedation in a day-hospital setting. All the aspirated specimens were obtained with a 22-gauge needle and were fixed in 10% formalin and paraffin embedded. Sections of 3 micron in thickness were cut and hematoxylin-eosin stained.

Results: From October 2005 to August 2016, 496 patients were submitted to EBUS-TBNA. Number of nodal stations punctured was 592 with a mean of 2.25 punctures per patient. Diagnosis of malignancy was obtained in 291 patients (58.6%). In 25 cases a nodal metastasis from an extrathoracic primary tumor was diagnosed. Sensitivity, specificity and diagnostic accuracy were 95%, 100% and 96% respectively. Negative predictive value was 90% and positive predictive value (PPV) was 100%. When molecular tests were requested, mutational analysis was successfully performed on cell block derived material in 55 out of 56 cases (98.2%), and fluorescence in situ hybridization (FISH) analysis in 26 out of 27 cases (96.2%).

Conclusions: EBUS-TBNA in our setting was an accurate and safe tool to diagnose non-lymphomatous nodal malignancies. Interestingly, in our series EBUS-TBNA has demonstrated to yield sufficient tissue for molecular analysis.

Keywords: Endobronchial ultrasound; lung cancer; transbronchial needle aspiration (TBNA); molecular analysis

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Introduction

Since introduction of ultrasound in bronchoscopy in 1992 (1) major technological advances have occurred. Endo-bronchial ultrasound (EBUS) has revealed useful in the assessment of the airway layer as well as in real time biopsies of lymph-nodes and central tumors in association with transbronchial needle aspiration (TBNA). Peripheral lung lesions are easily detected with radial EBUS mini-probes. New generation of ultrasound processors with Power/Color Doppler and elastography seem to be helpful in the prediction of metastatic lymph-nodes during EBUS-TBNA. In the last 10 years several studies reported high diagnostic accuracy, minimal invasiveness and wide accessibility to hilar and mediastinal nodes of EBUS-TBNA (2). For these reasons both American College of Chest Physicians (ACCP) and European Society of Thoracic Surgeons (ESTS) guidelines recommended ultrasound guided needle aspiration as the best first diagnostic tool to obtain tissue for the staging of lung cancer (3,4).

EBUS-TBNA has been shown also to be a valuable technique to diagnose isolated mediastinal metastases in patients with previous malignancies with accuracy of 97.7% (5). Role of trans-bronchial needle aspiration under ultrasound guidance in diagnosis of benign disease and haematological malignancies is controversial. In single centre studies results in diagnosis of lymphoma and sarcoidosis seem to be dependent on patient selection criteria, local cytopathology service and size of sample (6,7).

Herein we analyze ten years experience with EBUS-TBNA in our Thoracic Surgery Department at Fondazione IRCCS Ca' Granda University Hospital in Milan.

Methods

In our institution the first EBUS bronchoscope was available since 2005. Patients referred to our department with hilar and mediastinal lymph-nodes suspicious for malignancy were proposed for EBUS-TBNA after multidisciplinary meetings. Lymph-nodes with short axis diameter greater than 10 mm on contrast-enhanced computer tomography (CT) and/or metabolically active on positron emission tomography (PET) scan were considered as target for EBUS sampling.

Patients with high clinical suspect of lymphoma or sarcoidosis were not proposed for EBUS and scheduled for surgical biopsy.

Written informed consent was obtained from all patients before the procedure. All procedures were performed in a day-hospital setting. Topical local anaesthetic on vocal cords and oropharynx (lidocaine) was administered. Patients were placed under conscious sedation with midazolam and remifentanil by anesthesiologist on supine position, in association with nasal oxygen supplementation.

All procedures were performed using a flexible bronchoscope with linear convex probe with a frequency of 7.5 MHz (BF-UC160F-OL8, Olympus; Tokyo, Japan) and a dedicated 22-gauge needle (NA-201SX-4022, Olympus; Tokyo, Japan). Examinations were conducted in the operative theatre by a thoracic surgeon with the assistance of a resident or a nurse.

The nodal stations were classified according to the lymph node map of the 7th edition of TNM classification for lung cancer (8); staging of lung cancer nodes were sampled in order N3, N2 and N1 according to ESTS guidelines.

Each lymph node was punctured at least twice according to the quality of the specimen expelled from the needle: a long serpentine cast should be seen falling in the specimen collection system (*Figure 1*). In our experience no more than four passes were performed per nodal station in order not to last the procedure and cause discomfort to the patient.

Rapid-on-site cytology evaluation (ROSE) was available only in part of the cases and in the last 5 years was used less frequently due to the skill achieved from different operators (*Figure 2*).

All the aspirated specimens were fixed in 10% formalin and paraffin embedded. Sections of 3 micron in thickness were cut and hematoxylin-eosin stained. In differentiated primary lung cancer the diagnosis was performed only at looking hematoxylin-eosin. In all remaining cases an immunohistochemical examination was made to verify glandular differentiation obtaining a P40 and P63 expression. When a suspicious of neuroendocrine differentiation appeared on hematoxylin-eosin slides, immunohistochemical markers as CD56, Chromogranin A or Synaptophisin were used to confirm the diagnostic hypothesis. In patients with history of previous malignancies the specimens were investigated with immunohistochemical panels oriented to primary tumor.

When molecular tests were requested by oncologist in order to guide the therapeutic management of the patients, analyses were performed on cell block derived material.

For mutational analysis of epidermal growth factor receptor (EGFR), Kirsten rat sarcoma (KRAS), v-Raf murine sarcoma viral oncogene homolog B (BRAF)
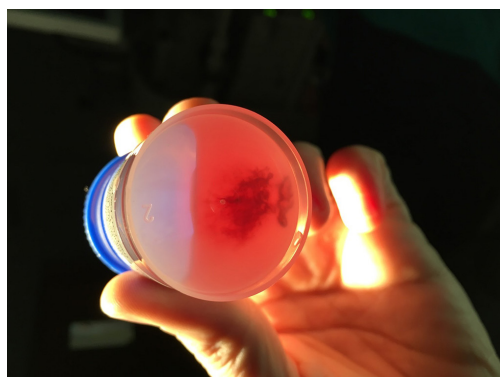
Figure 1 Long serpentine of specimen in the collection system.

Table 1 Nodal stations distributions and order of puncture

Nodal station	1st NS	2nd NS	3rd NS	Percentage
2L	2	–	–	0.3%
2R	5	–	–	0.8%
4L	24	3	–	4.5%
4R	191	6	–	33.4%
7	213	58	1	45.9%
10R	54	23	3	13.5%
10L	6	2	–	1.3%
11R	1	1	–	0.3%
Total number of punctures (592)	495	93	4	100.0%

NS, nodal station; L, left; R, right.

and neuroblastoma RAS viral oncogene homolog (NRAS), a ratio of tumor cells/non-neoplastic cells ≥10% was considered acceptable. Anaplastic lymphoma kinase (ALK) and c-ros oncogene 1 (ROS1) genes rearrangement tests and met proto-oncogene (MET) gene amplification test were performed in cases with at least 50 tumor cells.

EBUS-TBNA cytological results were assessed per patient. Inadequate specimen was defined in presence of an excess of red blood cells with no atypical or malignant cells identified, or if only bronchial cells were identified, or if the concentration of lymphocytes was not representative of lymph node tissue. The result of pathological examination was classified as negative when non-malignant cells (lymphocytes, histiocytes and anthracotic pigment) were detected in specimens.

Data are presented with numbers (percentages) for categorical variables and median [95% confidence interval (CI)] for continuous variables. The diagnostic sensitivity, specificity, positive predictive value (PPV), negative predicted value (NPV) and accuracy were calculated using standard definitions. A P-value <0.05 was considered significant. Statistical analyses were undertaken using MedCalc version 14.8 (Medcalc Software, Mariakerke, Belgium).

Results

From October 2005 to August 2016, a total of 496 patients underwent EBUS-TBNA. Among them 348 (70.1%) were men, with median age of 67 (range 16–85) years. Number of hilar and mediastinal nodal stations punctured were 592 with a mean of 2.25 punctures per patient. In 93 patients a second nodal station was punctured and a third station in 4 cases. The most common site of transbronchial aspiration was the subcarinal station (272, 45.9%), followed by the lower right paratracheal station (197, 33.2%) (*Table1*).

No major complication has been recorded after the procedure. Bronchospasm occurred in two cases (0.4%).

Overall, inadequate procedures were 76 (15.3%). Twenty-eight patients with inadequate results were scheduled for a surgical biopsy and 48 patients were admitted to other hospitals and proposed for a different diagnostic procedure.

Diagnosis of malignancy was obtained in 291 patients (58.6%). The two most common pathologic histotypes were adenocarcinoma (98, 19.7%) and unspecified non-small cell lung cancer (NSCLC) (101, 20.3%). Diagnosis of nodal metastasis from an extrathoracic primary tumor was made in 25 cases (*Table 2*). In 14 patients (2.8%) the diagnosis was granulomatous diseases: 12 sarcoidosis and 2 tuberculosis. The presence of lymphocytes in specimens without malignant cells determined 115 negative cases (23.1%). These results were confirmed by surgical biopsies or clinical follow-up (CT or PET scan) in 83 patients. There were 13 false-negatives and 8 patients were lost during follow-up.

Sensitivity, specificity and diagnostic accuracy were 95%, 100% and 96% respectively. Negative predictive value was 90% and PPV was 100%.

ROSE was performed in 258 cases (52%) with median procedure duration of 30 minutes (95% CI, 30.0–37.7). In 238 patients (48%) ROSE was not available mainly for technical reasons and in this group median procedure duration was 30 minutes (95% CI, 30–35). In our series
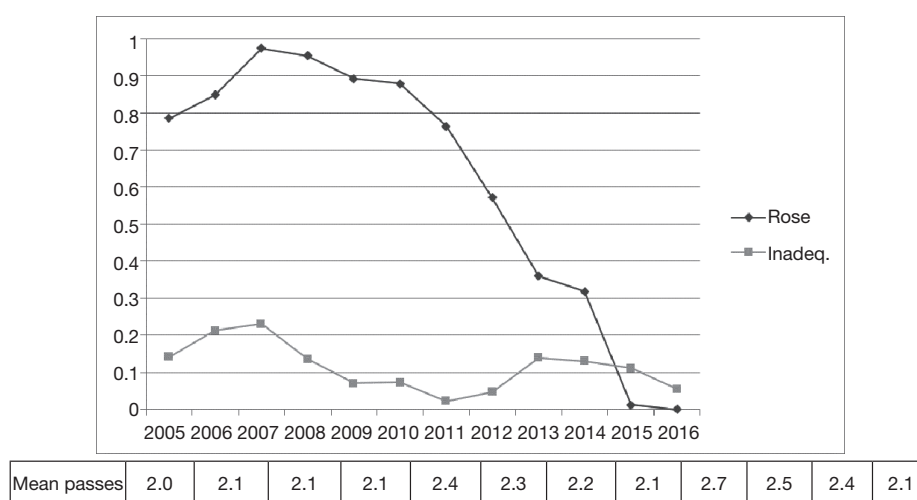
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| Mean passes | 2.0 | 2.1 | 2.1 | 2.1 | 2.4 | 2.3 | 2.2 | 2.1 | 2.7 | 2.5 | 2.4 | 2.1 |

Figure 2 Percentage of inadequate EBUS-TBNA per year and percentage of procedures done with rapid on-site evaluation per year. At bottom line mean needle passes per year.

Table 2 Pathological results of EBUS-TBNA

Diagnosis	Number	Percentage
Adenocarcinoma	98	19.7%
Squamous cell carcinoma	25	5.0%
Non-small-cell-carcinoma, unspecified	101	20.3%
Small cell lung cancer	3	0.6%
Neuroendocrine tumor	37	7.4%
Pleomorphic carcinoma	1	0.2%
Sarcoidosis	12	2.4%
Lymphoma	1	0.2%
Tuberculosis	2	0.4%
Other malignancies	25	5.0%
Renal cancer	4	–
Prostatic cancer	1	–
Colonic cancer	4	–
Hepatocellular carcinoma	2	–
Thyroid cancer	2	–
Melanoma	2	–
Ewing sarcoma	1	–
Gastric cancer	1	–
Salivary glands cancer	1	–
Breast cancer	6	–
Testicular seminoma	1	–
Negative	115	23.1%
Inadequate	76	15.3%
Total	496	100.0%

EBUS-TBNA, endobronchial ultrasonography with transbronchial needle aspiration.

we recorded no difference in terms of length of EBUS procedure conducted with or without ROSE (Chi-squared test P=0.50). ROSE defined the procedure as adequate in 199 patients (77.1%). In this subset of patients the final diagnosis was accurate in 90.5% of the cases; a non-accurate final diagnosis occurred in 2%, and in 7.5% of the cases specimens were finally judged as inadequate. Fifty-eight patients had ROSE inadequate result which ended in a final accurate diagnosis in 69% of the cases, a non-accurate diagnosis in 3.4% while a definitive inadequate cytological finding occurred in 27.6% (P<0.001). Considering the whole population (496 patients) a final inadequate result was obtained in 12% of patients who received ROSE and in 13.9% of patients who did not (P=0.82).

Molecular tests were requested in 54 NSCLC and 2 metastatic melanoma cases. Specifically, for the 52 NSCLC patients, the tests requested were: mutational status of *EGFR* (54 cases), *KRAS* (42 cases) and *BRAF* (39 cases), *ALK* rearrangement (27 cases), *ROS1* rearrangement (5 cases) and *MET* amplification (2 cases). For the two melanoma cases, *BRAF* and *NRAS* mutational test was requested.

Out of 56 EBUS cases, only one specimen was not molecularly characterized, because the specimen was depleted for the cytopathologic diagnosis (*Table 3*). All the remaining 55 specimens had an appropriate tumor cells component for the mutational analysis (tumor cells/non-neoplastic cells ≥10%), and the DNA amount obtained from each tissue was sufficient for the amplification reactions.

Table 3 Molecular analyses on EBUS-TBNA

Starting material	No. of cases	Cytopathological diagnosis	Mutational analysis method	Mutational analysis				FISH analysis		
				EGFR	*KRAS*	*BRAF*	*NRAS*	*ALK*	*ROS1*	*MET*
EBUS-TBNA	1	NSCLC	–	IN	–	–	–	–	–	–
	7	NSCLC	Pyr	P	–	–	–	–	–	–
	1	NSCLC	Pyr	P	P	–	–			
	4	NSCLC	Pyr	P	–	–	–	P	–	–
	18	NSCLC	MS	P	P	P	–	–	–	–
	2	Melanoma	MS	–	–	P	P	–	–	–
	16	NSCLC	MS	P	P	P	–	P	–	–
	2	NSCLC	MS	P	P	P	–	IN	–	–
	3	NSCLC	MS	P	P	P	–	P	P	–
	2	NSCLC	MS	P	P	P	-	P	P	P

P, successfully performed analyses; IN, inadequate specimens; –, tests not requested by the clinicians; MS, MALDI-TOF MS; Pyr, pyrosequencing. EBUS-TBNA, endobronchial ultrasonography with transbronchial needle aspiration; *EGFR*, epidermal growth factor receptor; *KRAS*, Kirsten rat sarcoma; BRAF, v-Raf murine sarcoma viral oncogene homolog B; *NRAS*, neuroblastoma RAS viral oncogene homolog; *ALK*, anaplastic lymphoma kinase; *ROS1*, c-ros oncogene 1; *MET*, met proto-oncogene.

Fluorescence *in situ* hybridization (FISH) test was requested for 27 EBUS cases, in all tissue specimens the tumor cellularity was appropriate for the analysis, as specified in the material and method section. In 25 out of 27 cases the analysis was completed, while in 2 cases the minimum tumor cells count for successful FISH testing was not achieved (*Table 3*), due to the consumption of the tissue specimens for the cytopathologic and the mutational analysis previously performed.

The mutational analysis performed on 53 NSCLC cases detected *EGFR* L858R mutation in 4 (7.5%) specimens. *KRAS* mutations were found in 5 out of 42 (11.9%) cases. Specifically, G12C and G13C mutations were seen in 2 cases, respectively, while G12V mutation was detected in 1 case. *BRAF* V600E mutation was detected in 1 out of 41 specimens.

One out of 27 cases was positive for *ALK* rearrangement (3.7%); *MET* amplification was seen in 1 out of 2 specimens, while all the 6 cases tested for *ROS1* rearrangement were negative.

Regarding the two metastatic melanoma specimens, *BRAF* V600E mutation was detected in one case, while *NRAS* was not found mutated. The molecular results are reported in *Table 4* and *Table 5*.

Overall, our data showed that the mutational analysis was successfully performed in 55 out of 56 EBUS specimens (98.2%), and FISH analysis in 26 out of 27

cases (96.2%). Only one specimen was completely depleted for cytopathologic diagnosis, not allowing the molecular characterization, and 3 out of 30 cases had no sufficient tumor cells number for appropriate FISH test.

Discussion

EBUS-TBNA in the past 10 years has become the method of choice in the staging of lung cancer and in the diagnosis of hilar and mediastinal lymphadenopathy. The main reason is that EBUS-TBNA is a safe procedure with high diagnostic yield that does not require general anesthesia and can be performed in an outpatient setting. In meta-analysis that includes 11 studies and 1,299 patients EBUS had a pooled diagnostic sensitivity of 93% and a pooled specificity of 100% (2). The diagnostic accuracy is lower in studies where patients are not selected on the basis of CT scan or PET scan positive results.

The availability of ROSE during EBUS-TBNA seems to reduce the rate of non-diagnostic sampling, the need for additional punctures and seems to increase sensitivity of the procedure (9,10). Even without rapid on-site cytological evaluation in several papers are reported diagnostic yield from 88% to 100% (11). A retrospective analysis on 131 patients underwent EBUS-TBNA with ROSE

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Table 4 Distribution of the mutations found in NSCLC and metastatic melanoma specimens

Molecular test	NSCLC		Metastatic melanoma	
	No. of cases	No. positive	No. of cases	No. positive
EGFR	53	4	–	–
KRAS	42	5	–	–
BRAF	41	1	2	1
NRAS	–	–	2	0
ALK	25	1	–	–
ROS1	5	0	–	–
MET	2	1	–	–

NSCLC, non-small cell lung cancer; EGFR, epidermal growth factor receptor; KRAS, Kirsten rat sarcoma; BRAF, v-Raf murine sarcoma viral oncogene homolog B; NRAS, neuroblastoma RAS viral oncogene homolog; ALK, anaplastic lymphoma kinase; ROS1, c-ros oncogene 1; MET, met proto-oncogene.

Table 5 Type of EGFR, KRAS and BRAF mutations detected in NSCLC and metastatic melanoma specimens

Mutation type	NSCLC		Metastatic melanoma	
	No. of cases	No. positive	No. of cases	No. positive
EGFR L858R, exon 21	53	4	–	–
KRAS G12C, exon 2	42	2	–	–
KRAS G12V, exon 2	42	2	–	–
KRAS G13C, exon 2	42	2	–	–
BRAF V600E, exon 15	41	1	2	1

NSCLC, non-small cell lung cancer; EGFR, epidermal growth factor receptor; KRAS, Kirsten rat sarcoma; BRAF, v-Raf murine sarcoma viral oncogene homolog B.

demonstrated that a final diagnosis was obtained in more than 73% of inadequate ROSE (12) and also in our series in 69% of inadequate ROSE an accurate diagnosis was obtained. On the contrary, even though the ROSE finding was adequate the final cytological result was inadequate in 7.5% of the cases. For those reasons we progressively reduced the use of ROSE during EBUS procedure without increasing the rate of inadequate cases (*Figure 2*).

We did not use EBUS-TBNA with the purpose of re-staging the mediastinum after induction therapy in lung cancer because diagnostic sensitivity is low (76%) and a NPV is unacceptable (20%) (13). After neoadjuvant chemotherapy to re-stage the nodal status we perform a surgical biopsy either by mediastinoscopy or by thoracoscopy.

Currently two different types of needle are available, 22-gauge and 21-gauge. A recent retrospective database analysis showed that there is no difference in specimen adequacy or diagnostic yield using needle with different diameters (14). When the procedure is performed with a 21-gauge needle adequacy with ROSE is achieved with less number of puncture. In our experience we prefer 22-gauge needle because the bronchoscope is more flexible and anatomical difficult nodal stations are easily reached.

The current standard of care for advanced lung cancer needs to identify key driver mutations and EBUS-TBNA has demonstrated to provide sufficient tissue for molecular testing (15). In our series the results are similar to those published in literature even if we do not have ROSE availability for all cases.

The EBUS-TBNA is a well tolerated procedure, complications are rare and similar to standard bronchoscopy. Major complications are reported with a rate lower than 0.1% (2). The bleeding complication is much less likely

with EBUS-TBNA then blind TBNA as the procedure is performed with real-time visualization of the needle.

Conclusions

In our experience EBUS-TBNA is a safe, accurate and well tolerated procedure with high diagnostic yield to diagnose intrathoracic malignancies. In the era of targeted therapies for NSCLC, EBUS-TBNA remains the first choice tool for diagnosis, staging and treatment planning especially in lung cancer patients.

Acknowledgements

None.

Footnote

Conflicts of Interest: The authors have no conflicts of interest to declare.

Ethical Statement: This retrospective observational cohort study has been approved by Ethics Committee of Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico in Milan. Written informed consent was obtained from all patients before the procedure.

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