Usefulness of conventional transbronchial needle aspiration in the diagnosis, staging and molecular characterization of pulmonary neoplasias by thin-prep based cytology: experience of a single oncological institute

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Background: Conventional transbronchial needle aspiration (c-TBNA) contributed to improve the bronchoscopic examination, allowing to sample lesions located even outside the tracheo-bronchial tree and in the hilo-mediastinal district, both for diagnostic and staging purposes.

Methods: We have evaluated the sensitivity, accuracy, positive predictive value (PPV) and negative predictive value (NPV) of the c-TBNA performed during the 2005–2015 period for suspicious lung neoplasia and/or hilar and mediastinal lymphadenopathy at the Thoracic endoscopy of the Thoracic Surgery Department of the Regina Elena National Cancer Institute, Rome. Data from 273 consecutive patients (205 males and 68 females) were analyzed.

Results: Among 158 (58%) adequate specimens, 112 (41%) were neoplastic or contained atypical cells, 46 (17%) were negative or not diagnostic. We considered in the analysis first the overall period; then we compared the findings of the first [2005–2011] and second period [2012–2015] and, finally, only those of adequate specimens. During the overall period, sensibility and accuracy values were respectively of 53% and 63%, in the first period they reached 41% and 53% respectively; in the second period sensibility and accuracy reached 60% and 68%. Considering only the adequate specimens, sensibility and accuracy during the overall period were respectively of 80% and 82%; the values obtained for the first period were 68% and 72%. Finally, in the second period, sensibility reached 86% and accuracy 89%. Carcinoma-subtyping was possible in 112 cases, adenocarcinomas being diagnosed in 50 cases; further, in 30 cases molecular predictive data could be obtained.

Conclusions: The c-TBNA proved to be an efficient method for the diagnosis/staging of lung neoplasms and for the diagnosis of mediastinal lymphadenopathy. Endoscopist's skill and technical development, associated to thin-prep cytology and to a rapid on site examination (ROSE), were able to provide by c-TBNA a high diagnostic yield and molecular predictive data in advanced lung carcinomas.

Introduction

Conventional transbronchial needle aspiration (c-TBNA) has contributed to implement the bronchoscopic examination, allowing to sample lesions located even outside the tracheo-bronchial tree and in the hilo-mediastinal district. In addition to the original diagnostic value, the flexible bronchoscopy has also allowed the staging of pulmonary neoplasms. Although the first description of a transbronchial sampling of a mediastinal lymph node was reported in 1949 (1), the "father" of c-TBNA should be considered Ko-Pen Wang of the Baltimora J. Hopkins University who in the 80s reported on the safety and usefulness of c-TBNA in N staging of lung tumors and in the diagnosis of hilo-mediastinal masses (2,3). However, for several years, this diagnostic approach was inconsistently applied due to the limited diagnostic yield, to the fear of complications and also to the needle costs. On the basis of available evidences, the c-TBNA should be considered safer than other bronchoscopic sampling methods, such as the transbronchial biopsy, widely used among thoracic endoscopists (4). In addition, a positive cost-effectiveness of c-TBNA has been described with comparison to the traditional surgical lung neoplasm staging techniques [mediastinoscopy, mediastinotomy, video-assisted thoracoscopic surgery (VATS)] mainly if the c-TBNA is supported by the presence of a pathologist performing a "rapid on site examination" (ROSE) (5,6). Still in the last 15 years, the endobronchial ultrasound-guided transbronchial needle aspiration (EBUS-TBNA) has been developed, based on a echografic guide situated on the bronchoscope, allowing both the needle visualization while crossing the bronchial wall and sampling of the mediastinal structure(s). The findings with the echoendoscope show sensibility of 94–95.7% independently from the size and location of the sampled lymph nodes (7-9). Thus, the aim of this study was to assess and discuss if the c-TBNA, associated to thin-prep based cytology, still maintains an important role in the diagnosis and in the staging of advanced lung neoplasias, on the basis of the findings of a single oncological institute.

Methods

All cases which performed the c-TBNA during the period 2005–2015 for suspicious or advanced lung neoplasia on the basis of computed tomography (CT), at times associated to positron emission tomography-CT (CT-PET), have been sorted out from the files of the bronchoscopic archives at the Thoracic endoscopy of the Thoracic Surgery of the Regina Elena National Cancer Institute, Rome, Italy. Data from 273 consecutive patients (205 males and 68 females) were collected. The demographical information and both the bronchoscopic and pathological reports have been used to establish a complex database (DB). Different additional cyto-histological approaches including bronchial brushing, bronchial washing and biopsy have been applied in association to c-TBNA in 47 cases; transbronchial biopsy has been performed subsequently in dubious cases, sometimes together. The International Association for the Study of Lung Cancer (IASLC) lymph node map was used to nominate the Lymph node stations (10). Molecular analyses were performed on the samples with "adenocarcinoma" and "probably adenocarcinoma" diagnosis. An informed consent has been signed by patients to the bronchoscopic procedure. The IRCCS Lazio Central Ethics Committee reviewed and approved the study (R.S. 833/16). Individual consent for the study was waived because the patients remained anonymous.

Bronchoscopy procedure

In the first period [2005–2011], the bronchoscopic procedure has been performed in local anaesthesia of the upper respiratory tract and light sedation with 3 mg midazolam. In the second period [2012–2015], the procedure was associated to deep sedation with a small bolus injection of anesthetic propofol and fentanyl in bolus or remifentanil in continuous infusion. As a routine procedure the c-TBNA was performed in a day hospital setting. A flexible videobronchoscopic (Olympus BFT160) was applied per os. Vital parameters were controlled and O2 was administered

by a nasal cannula in order to maintain a 90% oxygen saturation. An accurate preliminary workup was performed by CT with contrast to identify the c-TBNA target and its relation to the tracheobronchial tree, and to apply correctly the needle. The c-TBNA was performed on hilar and mediastinal lymph nodes enlarged (short axis >2 cm) according to CT, suspicious for neoplasia. Two different fine needles of 21 Gauge (G) have been used: EXCELON 6410, Boston Scientific, and WANG MW-121, Conmed. Different passages were performed: at the beginning we performed one passage, between 2007–2011 at least two passages and in the period 2012–2015 we performed three or more passages. A single bronchoscopist (MF) with a longstanding expertise performed the c-TBNA procedure before any other bronchoscopic approach. In the last period, starting from March 2014 to December 2015, in 23 cases a dedicated pathologist (FM) assisted to the procedure in order to verify the adequacy of the material provided (ROSE). To be considered adequate (i.e., centred in a lymph node), the material should have been containing at least a few lymphocytes.

Cytological procedure

Since 2005, c-TBNA specimens have been treated with ThinPrep (Cytyc, Marlborough, MA, USA) process: the cytological specimen collection was placed directly into a methanol-based preservative solution (Cytyc CytoLyt); following centrifugation and discarding of the supernatant, the cell pellet was resuspended and a sample transferred to a second methanol-based preservative (Cytyc Preservcyt); subsequently, the ThinPrep processor yields an alcohol-fixed slide preparation. The processor disperses cells, collects them on a polycarbonate filter, and finally transfers the cells to a glass slide, which is then immersed in 95% alcohol and then stained with Papanicolaou technique.

Rapid on site staining technique (ROSE)

A fine needle aspiration (FNA) was placed onto a holder, and then divided into two aliquots, each of which was then transferred on a slide as a smear; specimen was fixed 20 sec in 95% ethanol, then stained in Harris hematoxylin; in contrast specimen stainings last for 30–45 sec, followed by rapid rinsing in water. Later, both samples were protected by a cover slide, without mounting medium and dried while removing any eventual air bubbles; finally, slides were examined under a light microscope.

Molecular analyses

Slides derived from cytological samples processed by the monolayer ThinPrep method and stained with Papanicolaou were reviewed by two pathologists with specific expertise in cytology (FM, PV) and samples with at least 50% neoplastic cells were used for molecular analysis. The Thin Prep material stored in the vial was used for DNA isolation performed with the QIAmp DNA kit (Qiagen) according to the manufacturer's instructions.

EGFR gene mutational analysis

In the period 2010–2012, exons 19–21 of *EGFR* gene and exon 2 of *KRAS* gene were PCR amplified by AmpliTaq Gold 360 Master Mix (Applied Biosystems, Foster City, CA, USA). All PCR products were directly sequenced with the V3.1 Big Dye Terminator Cycle Sequencing Reaction Kit (Applied Biosystems) and then run on ABI PRISM 3130 (Applied Biosystems). Clustaw2 alignment software EMBL-EBI was used to perform sequencing analysis.

Real-time PCR

In the period 2013–2015, *EGFR* (exon 18-19-20-21) and *K-RAS* (exon 2-3-4) mutation status was evaluated by a rapid and sensitive real time TaqMan assay method (EntroGen, Inc.) with EGFR/KRAS reporter FAM, quencher NFQ-MGB, control reporter VIC. Amplification and allelic discrimination were performed on an Applied Biosystems 7500 Real Time PCR system.

Fluorescence *in situ* hybridization (FISH) of ALK, ROS1 rearrangements

ALK rearrangements at 2p23 chromosome locus and *ROS1* rearrangements at 6q22 chromosome locus were investigated by FISH. To detect *ALK* translocation the ALK FISH DNA Probe 2p23 Split Signal Code Y5417 was used (Dako, Milan, Italy). To detect *ROS1* rearrangements ROS1 IQFISH Break-Apart Probe 6q22 Code G111601-8 was used (DAKO, Agilent Genomics). The results of the hybridization were interpreted according to the manufacturer's instructions and current guidelines on molecular testing (11-15).

Statistical analysis

Sensitivity, accuracy, positive predictive value (PPV) and negative predictive value (NPV) of the tests were calculated according to the standard definitions as follows: sensitivity = true positives/true positives + false negatives; accuracy

Table 1 Overall results of adequacy of c-TBNA in 273 patients by year

Adequacy	2005–2007	2008	2009	2010	2011	2012	2013	2014	2015	Total
No	21	13	8	7	9	21	11	11	14	115
Yes	22	15	5	10	12	21	18	21	34	158
Total	43	28	13	17	21	42	29	32	48	273

c-TBNA, conventional transbronchial needle aspiration.

Table 2 Diagnosis distribution in adequate specimens (158 cases) by year

Years	Adequate/non diagnostic*	Atypia	NSCLC Nos	Adenocarcinoma	SCC	SCLC	Metastasis	Total
2005–2007	10	3	4	–	1	4	–	22
2008	6	3	3	–	1	1	1	15
2009	3	1	–	–	1	–	–	5
2010	–	1	3	2	–	2	2	10
2011	6	–	2	3	1	–	–	12
2012	7	–	1	7	3	3	–	21
2013	–	1	1	10	1	5	–	18
2014	6	–	–	10	1	4	–	21
2015	8	–	–	18	2	6	–	34
Total	46	9	14	50	11	25	3	158

*, neoplastic cases diagnosis with additional methods. NSCLC Nos, non small cell lung carcinoma not otherwise specified; SCC, squamous cell carcinoma; SCLC, small cell lung carcinoma.

= true positives + true negatives/total population; PPV = true positives/true positives + false positives; NPV = true negatives/true negatives + false negatives. Data were reported as mean ± standard deviation (SD). The confidence intervals have been calculated with the following formula: (accuracy, PPV, NPV) ±1. 96× standard error (SE). The probability area between the points of abscissa –1.96 and +1.96 is 0.95. A z_i point in a standard normal distribution has the probability of 95% to fall within the range considered, and 5% of falling outside. Microsoft Excel was used for data entry and statistical analysis.

Results

Among the 273 total cases (205 patients were males, 68 were females, mean age 65±11 y) with suspected lung cancer and involvement of mediastinal lymph nodes, 158 (58%) c-TBNA samples have been considered adequate for diagnostic purposes. In the remaining 115 (42%) cases a diagnosis was not possible because the samples were considered inadequate (i.e., containing only bronchial normal epithelial cells and/or red cells) (*Table 1*). Among

158 adequate specimens, 112 were frankly neoplastic or with atypical cells, 46 were negative for malignant/atypical cells, four out of the 46 negative cases proved to be positives for malignancy on subsequent biopsy (false negative). The most frequent histological type was adenocarcinoma (32%), followed by small cell lung carcinoma (SCLC) (16%), non small cell lung carcinoma (NSCLC), NOS (9%) and squamous cell carcinoma (SQCC) (7%); 9 patients had atypical cells, (epithelial and/or lymphoid) and 3 patients had metastatic tumours (2 from breast cancer, 1 from melanoma) (*Table 2*).

The number of the procedures performed varied along the years; in the first three years, only 43 procedures were performed, on the whole; therefore we report overall data for years 2005–2007. A relative decrease in the years 2009–2011 was related to the contemporary initial activity of the endoscopic ultrasound trans-esophageal FNA (EUS-FNA) on the posterior mediastinal lymph node stations, performed at the Gastrointestinal Endoscopy Department. A strong increase in adequacy has to be noticed in the period 2013–2015. Therefore we considered the sensibility, accuracy and PPV and NPV on the overall period (*Table 3*)

and in the two periods separately (2005–2011 and 2012–2015) (*Table 4*). During the overall period, sensibility and accuracy values were respectively of 53% and 63% (*Table 5*). In the first period [2005–2011] sensibility and accuracy were 41% and 53% respectively; in the second period [2012–2015] sensibility and accuracy raised to 60% and 68% (*Table 6*). The PPV always reach 100%; NPV, rather low at the beginning (31%), in an evaluation of the two periods together or in the second period reached the value of 38%. Considering only the 158 adequate specimens, sensibility and accuracy during the overall period were respectively of 80% and 82%; in the first period [2005–2011] sensibility and accuracy reached 68% and 72%; finally, in the second period sensibility reached 86% and accuracy 89% (*Table 6*).

In the overall period 2005–2015, the lymph node stations by frequency have been the subcarinal (station 7: 69 cases), the superior mediastinal (3A, 4R) and together the hilar (total: 63 cases), hilar-lobar (station 11: 36 cases), superior mediastinal and subcarinal together (3A and 7, 27 cases) right and left hilar principal (10R 26 cases and 10L 26 cases); 21 peribronchial tumors were also investigated by c-TBNA, 11 out of 21 samples being positive (*Table 7*). The higher sensitivity was achieved for the station 11, followed by the subcarinal station 7 and by the station 7 together with the 3A station (*Table 7*). Among the

112 cases adequate and positive for neoplasia, 65 cases had the diagnosis exclusively with the c-TBNA, in 47 cases other cyto-histological endoscopic procedures were associated. Therefore the c-TBNA alone was diagnostic in variable percentage of cases, with a peak of 59% in 2010 and 62% in 2015; during the overall period, 43 cases were lost to follow up and it was not possible to establish the diagnosis. The results derived from c-TBNA in comparison with the final diagnosis were shown in *Table 8*.

Molecular genetic determinations: in the period 2010–2015 in 30 out of 50 adenocarcinoma/likely adenocarcinoma cases a molecular investigation has been successful, in the first two years by investigating only the *EGFR* gene. Later, by sequential analyses, other relevant genes were also investigated: K-*RAS*, *ALK* and *ROS*1. On the whole, 48 single and/or sequential evaluation of multiple genes were performed. *Table 9* shows the percentages of the molecular determinations compared to the number of patients affected by adenocarcinoma. In the overall period an average of 60% of cases were tested by molecular biology, with a maximum of 80% in 2013.

Discussion

As clearly demonstrated from the literature, c-TBNA results are largely influenced by several parameters: lymph node target size (>2 cm), passage number, the improvement in the endoscopist's skill, the needle size and the presence of a pathologist on site (16,17).

We collected and analyzed the c-TBNA data from a series of 273 patients with suspect or advanced lung cancer. The patient series has been analysed in two periods, 2005–2011 and 2012–2015. The size of the lymph nodes varied between 2 and 6 cm, the c-TBNA finding the prevalent indication when the suspected lymph nodes had a diameter >2 cm. The passage number varied along the years: as also shown in the literature (17), being single in the first 2 years [2005–2006], double in the period 2007–2011 and at least triple (until a maximum of five passages) in the period 2012–2015.

Table 3 Overall patients [273]—sensitivity, diagnostic accuracy, and predictive values obtained by c-TBNA, 2005–2015

Measures	No. of patients	Percentage and 95% CI
Sensitivity	112/(112+100)	53 [46–60]
Accuracy	(112+61)/273	63 [58–69]
PPV	112/(112+0)	100
NPV	61/(100+61)	38 [30–45]

c-TBNA, conventional transbronchial needle aspiration; PPV, positive predictive value; NPV, negative predictive value; CI, confidence interval.

Table 4 Overall patients by period—sensitivity, diagnostic accuracy, and predictive values obtained by c-TBNA, 2005–2011 and 2012–2015

Measures	2005–2011		2012–2015	
	No. of patients	Percentage and 95% CI	No. of patients	Percentage and 95% CI
Sensitivity	39/(39+57)	41 [31–51]	73/(73+48)	60 [52–69]
Accuracy	(39+26)/122	53 [44–62]	(73+30)/151	68 [61–76]
PPV	39/(39+0)	100	73/(73+0)	100
NPV	26/(57+26)	31 [21–41]	30/(48+30)	39 [28–49]

c-TBNA, conventional transbronchial needle aspiration; PPV, positive predictive value; NPV, negative predictive value; CI, confidence interval.

Accordingly, in the first 2 years the sensibility was of 41%, with 57 cases out of 122 inadequate, and accuracy was of 53%. In the second period every single lymph node station has been stinged at least 3 times until to a maximum of 5 passages, and the sensibility raised to 60% with a parallel decrease of inadequate samples (48 out of 151 samples) and the accuracy reached the 68%. The hilar stations (N1) have been always reached with high sensibility and accuracy, whereas the mediastinal stations (N2) at the beginning were often non diagnostic or no accuracy was reached; in the second period 2012–2015, however, the sampling of the N2 stations became very often diagnostic. The best results were obtained on the lymph node stations 11, 7 e 3A, in relation with the best endobronchial landmarks available for the optimal aspiration site. A crucial point is the endoscopist's experience: the technique in itself is simple and it is possible to learn "by the book" and with exercise on inert models when no formal training is possible (18). Literature data point on the necessity of at least 50 procedures, assisted by an experienced endoscopist (19-24). In our study the percentage of adequate cases rose from 52% in the first period to 62% in the second period. Similar results had been reported by other studies (25). Needle size doesn't appear to be relevant in order to ensure the diagnostic yield: several studies demonstrated that hilar and mediastinal lymph nodes with relevant neoplastic involvement may be sampled both with needle for cytology (21 G) and with needle for histology (19 G). For suspicious lymphoma or granulomatous disease the needle providing the best

Table 5 Adequate specimens [158]—sensitivity, diagnostic accuracy, and predictive values obtained by c-TBNA, 2005–2015

Measures	No. of patients	Percentage and 95% CI
Sensitivity	112/(112+28)	80 [73–87]
Accuracy	(112+18)/158	82 [76–88]
PPV	112/(112+0)	100
NPV	18/(28+18)	39 [25–53]

c-TBNA, conventional transbronchial needle aspiration; PPV, positive predictive value; NPV, negative predictive value; CI, confidence interval.

Table 6 Adequate specimens by period—sensitivity, diagnostic accuracy, and predictive values obtained by c-TBNA, 2005–2011 and 2012–2015

Measures	2005–2011		2012–2015	
	No. of patients	Percentage and 95% CI	No. of patients	Percentage and 95% CI
Sensitivity	39/(39+16)	71 [56–80]	73/(73+12)	86 [80–94]
Accuracy	(39+7)/64	72 [61–83]	(73+11)/94	89 [82–95]
PPV	39/(39+0)	100	73/(73+0)	100
NPV	7/(16+7)	30 [10–46]	11/(12+11)	48 [26–69]

c-TBNA, conventional transbronchial needle aspiration; PPV, positive predictive value; NPV, negative predictive value; CI, confidence interval.

Table 7 Sensitivity obtained by the total 273 c-TBNA specimens in different nodal stations [classified according to IASCL node map (10) and in 21 peribronchial tumors sampled]

Nodal station	No. of patients	TBNA positive for cancer	TBNA negative for cancer	Sensitivity (%)
7	69	35	34	51
11	36	19	17	53
10R	26	10	16	38
10L	26	7	19	27
3A and 7	27	13	14	48
3A, 4R and 10L	63	16	47	25
7, 3A and 4R	5	1	4	20
Peribronchial tumor	21	11	10	52
Total	273	112	161	41

c-TBNA, conventional transbronchial needle aspiration.

Table 8 Percentage of diagnoses exclusively with the c-TBNA *vs.* other cyto-histological endoscopic procedures

Year	Other cytology and/or histology (%)	c-TBNA (%)	No diagnosis (%)	Total
2005–2007	25 (58%)	12 (28%)	6 (14%)	43
2008	15 (54%)	9 (32%)	4 (14%)	28
2009	6 (46%)	1 (8%)	6 (46%)	13
2010	6 (35%)	10 (59%)	1 (6%)	17
2011	13 (62%)	6 (29%)	2 (10%)	21
2012	22 (52%)	14 (33%)	6 (14%)	42
2013	9 (31%)	18 (62%)	2 (7%)	29
2014	13 (41%)	15 (47%)	4 (13%)	32
2015	9 (18%)	27 (56%)	12 (25%)	48
Total	118 (43%)	112 (41%)	43 (16%)	273

c-TBNA, conventional transbronchial needle aspiration.

Table 9 Percentage of cases with molecular test (patients with molecular tests/patients with adenocarcinoma or probable adenocarcinoma)

Year	No. of patients	EGFR	EGFR, ALK	EGFR, RAS	EGFR, RAS, ALK	EGFR, RAS, ALK, ROS1	ROS1, ALK	Total (%)
2010	3	1	–	–	–	–	–	1/3, 33%
2011	2	1	–	–	–	–	–	1/2, 50%
2012	7	1	–	1	–	–	–	2/7, 30%
2013	10	5	2	1	–	–	–	8/10, 80%
2014	10	1	1	1	1	1	1	6/10, 60%
2015	18	6	2	4	–	–	–	12/18, 67%
Total	50	15	5	7	1	1	1	30/50, 60%

EGFR, epidermal growth factor receptor; ALK, anaplastic lymphoma kinase; RAS, rat sarcoma gene; ROS-1, ROS proto-oncogene 1, receptor tyrosine kinase.

diagnostic yield (19 G) should be used after adequate training and experience of the endoscopist with fine needle 20–21 G (16).

According to the Guidelines of the American College of Chest Physicians (26), the presence on site of a pathologist is not recommended: however, in our experience, the pathologist presence improve the diagnostic yield and decreases the costs, either for the material reduction either by avoiding further investigations. In our study, in fact, the adequacy reached the 71% in the last year, when we adopted the ROSE. The results obtained with regard to sensibility and accuracy were comparable to those reported with the help of echography (EBUS-TBNA) (26-28).

In our experience we didn't observed complications, such as described in the literature, neither anaesthesiologic problems. Actually the rare complications associated to c-TBNA include fever, slight bleeding and bacterial pericarditis. Moreover mediastinitis and pneumothorax

have been described, usually with spontaneous and favorable resolution (29,30).

The recent revision of adenocarcinoma classification in different subtypes proved to be clinically relevant; different genic profiles have been associated to the histologic subtypes, bearing prognostic and predictive value (31-34). Molecular testing of lung adenocarcinoma for the epidermal growth factor receptor (*EGFR*) and anaplastic lymphoma kinase (*ALK*) is now considered standard of care and part of the diagnostic algorithm together with a search for other "driver mutations" in oncogenes such as *KRAS, ROS1* (11). *EGFR* mutations are present in approximately 15% of primary lung adenocarcinomas and are mutually exclusive of *KRAS* and *ALK* mutations (35). Rearrangements of *ROS1* appear mutually exclusive of other known oncogenic drivers (https://www.mycancergenome.org/content/disease/lung-cancer/ros1/67/).

At the moment predictive molecular tests are usually

performed on the primary tumor (T). Although the first studies correlating genetic alterations in metastatic lymph nodes have been deluding (36-38), other reports demonstrated that primary tumor mutational status not always correlate with the metastatic lymph node genetic status (39). Chen *et al.* showed that the therapeutic response to tyrosine kinase inhibitor (TKI) acting on EGFR is more pronounced in patients with mutated tumor in the metastatic foci (40): therefore the mutational status of the N component could be more predictive of therapy response than that of the primary tumor (41).

Although in the literature it is not possible to compare the efficacy of c-TBNA *vs.* EBUS-TBNA in molecular studies, it has been shown that the number of passages improves the diagnostic yield (42). In our study the molecular diagnostic yield increased in parallel with the c-TBNA sensibility and accuracy, the samples being sufficient to provide molecular data in 60% of adenocarcinoma samples, with a relevant increase from 2010 (33% of adequate cases) to 2015 (67% of cases effective in molecular characterization), with a maximum of 80% in 2013. The availability of the thin-prep technique offered a consistent improvement of diagnosis and provided material for molecular analyses, comparable with the results obtained by histological samples/biopsies (43-45).

Finally in our study the c-TBNA was the first and unique diagnostic procedure in 41% of total cases (65/158), avoiding major, invasive and expensive diagnostic procedures. Associated to other diagnostic procedures (brushing, BAL, biopsy) the c-TBNA proved to be diagnostic in 71% of cases (112/158).

Conclusions

Our study show demonstrated that the c-TBNA today still represents an efficient method for diagnosis and staging of advanced lung tumours (46,47). The diffusion of EBUS certainly improved the sensibility, however, whenever the more advanced technologies are not available, the c-TBNA represents a milestone in diagnostics (48). Moreover, the initial phase of EBUS setting and the needles are very expensive. Therefore only referral centers, at the moment, can afford these expenses. The c-TBNA could be performed also during the first diagnostic bronchoscopy in case of suspicious lung tumour and it could represent the first and unique diagnostic procedure, if positive. More complex or more invasive procedures are indicated whenever the c-TBNA doesn't provide the diagnosis. EBUS is indicated as first diagnostic procedure in case of lymph node diameter < to 2 cm or in not easily reachable sites, such as stations 2R, 2L, 4L or lower subcarinal. Therefore c-TBNA is a complex but affordable procedure to be performed, with low costs, even in peripheral but well trained centers of thoracic expertise.

Acknowledgements

Footnote

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

Ethical Statement: The study was approved by the IRCCS Lazio Central Ethics Committee (No. R.S. 833/16). A written informed consent to the procedure was obtained from all patients.

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