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# Useful on-site macroscopic quantitative evaluation of EBUS-TBNA samples

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## Abstract

**Background:** Endobronchial ultrasound-guided transbronchial needle aspiration (EBUS-TBNA) is a minimally invasive diagnostic test with a high diagnostic yield. Optimal specimen acquisition is of a key importance; however, rapid on-site cytology examination (ROSE) during EBUS-TBNA has limited access. In an attempt to provide immediate feedback to the operator, the intervention pulmonology team started to classify macroscopically every lymph node sample in terms of abundance. The main objective of this study was to clarify whether this on-site macroscopic evaluation of EBUS sample was related to histology results and therefore any helpful for clinical practice.

**Results:** From a total of 288 lymph node stations sampled, adequate/diagnostic histological results were present in 11.1% of “insufficient” samples, 40.9% “sufficient” samples, 76.4% “good” samples and 88.1% of “excellent” samples. This was statistically significant with *p*-value under 0.0001. Also, there was a statistical agreement between samples that were macroscopically “insufficient” or “sufficient” with “inadequate” and “dubious” histological results and also between macroscopically “good” and “excellent” samples and histological results that were adequate/diagnostic. And that statistical agreement was of moderate magnitude according to the “Landis and Koch” classification ( $k = 0.386$ ).

**Conclusions:** The positive relationship between the on-site macroscopic evaluation of the EBUS sample as “excellent” and the “adequate/diagnostic” histological result is of high clinical importance since it represents a fast, cheap and easy method that provides immediate feedback to the operator influencing his conduct; by obtaining additional passes to achieve an “excellent” will ensure a higher percentage of samples with a diagnosis, accelerating the patients treatment.

**Keywords:** EBUS-TBNA, On-site quantitative macroscopic evaluation, ROSE, Lung cancer, Adenopathies, Granulomatous diseases

## Background

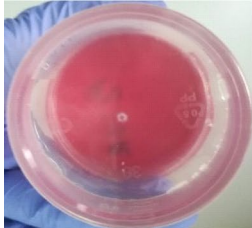
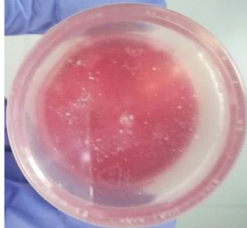
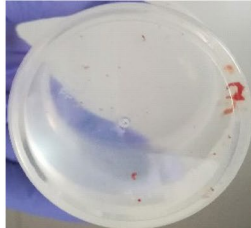
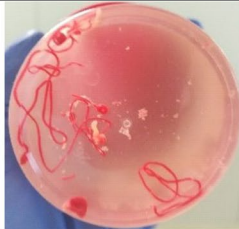
Endobronchial ultrasound-guided transbronchial needle aspiration (EBUS-TBNA) is a minimally invasive diagnostic test with a high diagnostic yield. In this procedure, abundance of material and optimal specimen acquisition is crucial for obtaining adequate tissue for diagnosis. In an attempt to increase diagnostic yield [1], there have

been studies associating rapid on-site cytology examination (ROSE) during EBUS-TBNA, which might be useful, however with limited access [2, 3]. Based on a similar concept to ROSE of providing immediate feedback to the operator and therefore increasing diagnostic yield and for not having access to it in the institution, the endoscopic team started to classify macroscopically every lymph node sample in terms of abundance. To the authors' knowledge, to date, there is no quantitative measure of EBUS-TBNA samples. The main objective of this study was to clarify whether this on-site macroscopic evaluation of EBUS sample abundance was related to histology

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**Table 1** Macroscopic evaluation of EBUS-TBNA samples

	Insufficient	Sufficient	Good	Excellent
Description	No visible material	Blurred saline or Few small fragments	One filament or Multiple small fragments	Multiple filaments
Example				

**Table 2** Histological classification of samples

Classification	Description
Inadequate	Containing a preponderance of bronchial cells, a minority or no lymphocytes, and no findings specific to a diagnosis
Dubious	Minority of lymphocytes that might correspond to blood contamination
Adequate/positive	Containing a preponderance of lymphocytes or containing features specific to a diagnosis

results, namely lymph node representativeness or disease diagnosis and therefore any helpful for clinical practice.

**Methods**

Retrospective study of patients undergoing EBUS-TBNA in the year of 2018. All procedures were performed with an EBUS bronchoscope (BF-UC180F Olympus) and Olympus ViziShot 22-g needle. The procedures were monitored and performed under general anaesthesia or mild sedation after patients signed a written informed consent. The lymph nodes to be sampled were left up to the discretion of each operator, who made a decision based on image, association with higher cancer staging and/or ease of sampling. At least three needle passes were conducted for each location as recommended [4]. Fragments of tissue were removed gently and put in a formalin solution, and assessment was made on cell block. The fragments were evaluated as “insufficient”, “sufficient”, “good” and “excellent” according to material abundance as exemplified in Table 1.

Patient demographic variables, procedure variables and histologic reports were analysed. Because there is no “gold standard” for EBUS specimen adequacy and being aware of cellular “contaminants” that are acquired when the needle passes through the tracheal wall en route to the targeted lesion, authors classified histological results as “inadequate”, “dubious” or “adequate/diagnostic” as described in Table 2.

**Table 3** Excluded EBUS procedures

Excluded procedures	n
Biopsy of other structure rather than a lymph node	6
Vascular interposition that enables the biopsy	4
The lesion was in fact a cyst	3

Statistical analyses were made by SPSS; comparisons were performed with chi-square test and agreement by Cohen’s K-test. Agreement’s magnitude was classified according to Landis and Koch classification. All tests were two sided, and a *p*-value < 0.05 was considered to be statistically significant.

**Results**

One-hundred thirty-four EBUS-TBNA were conducted in the year of 2018; 13 exams were excluded for corresponding solely to biopsies of structures other than lymph nodes or exams in which biopsy was not possible as described in Table 3. A total of 121 procedures were included, corresponding to 117 patients.

Of the 117 patients included, 68.37% (*n* = 80) were male and 31.62% were female (*n* = 37). The mean age was 64.1 years (*SD* 11.1 years).

The indications for EBUS-TBNA conducted in 2018 were lung micronodules/nodules and adenopathies in 24 exams (19.84%), lung mass and adenopathies in

**Table 4** Motives for EBUS execution

		n	%
Without previous diagnose	Lung micronodules/nodules and adenopathies	24	19.84
	Lung mass and adenopathies	26	21.49
	Isolated adenopathies	22	18.18
With previous diagnose	Cancer staging	28	23.14
	Suspicion of cancer recurrence	21	17.35
Total		121	100

26 exams (21.49%), isolated adenopathies in 22 exams (18.18%), cancer staging in 28 exams (23.14%) and suspicion of cancer recurrence in 21 exams (17.35%) (Table 4). Only 7 procedures (5.7%) were made under mild sedation with midazolam and 114 (94.3%) under general anaesthesia. During these procedures, a total of 288 lymph node stations were sampled. The median number of lymph node stations biopsied per procedure was 2.1. The median number of lymph node stations biopsied per procedure under mild sedation with midazolam was 1.5 and 2.18 with general anaesthesia. There was at least 1 station punctured and a maximum of 5 stations punctured in the procedures analysed. The mean number that each lymph node station was punctured was of 3.81 times, and there was no significant difference in the number of punctures of lymph nodes' samples classified as "insufficient" (3.33 times), "sufficient" (3.63 times), "good" (3.56 times) or "excellent" (3.59 times).

Location of the lymph nodes biopsied according to the IASLC classification [5] is described in Table 5.

The most common lymph node station biopsied was station 7 (34.03%), followed by station 4R (26.02%) and station 11R (18.06%). No statistical difference was found between the station number and histological adequacy, since some stations were rarely punctured.

By cross tabulation, macroscopically "insufficient" samples were mostly inadequate ( $n = 9/88.9\%$ ), and only 1 sample was adequate/diagnostic (1.1%) in the histological classification. Of the 22 macroscopically "sufficient" samples, 3.8% were inadequate, 27.5% were dubious and 40.9% were adequate/diagnostic by histological classification. From the 55 macroscopically "good" samples, 20.0% were inadequate and 76.4% adequate. And finally, from the 178 macroscopically "excellent" samples, 88.1% were adequate/diagnostic, only 6.4% were dubious, and 5.4% were inadequate by histological classification. This was statistically significant with  $p$ -value under 0.0001. This is described in Table 6.

**Table 5** Location of the lymph nodes biopsied according to the IASLC classification

Location of the lymph nodes biopsied		
Station	n	%
2L	2	0.69
2R	2	0.69
3p	1	0.36
4L	25	8.68
4R	75	26.02
7	98	34.03
10L	4	1.39
10R	4	1.39
11L	21	7.29
11R	52	18.06
12L	1	0.36
12R	3	1.04
Total number of stations biopsied	288	100

Moreover, there was a statistical concordance between samples that were macroscopically "insufficient" or "sufficient" with "inadequate" and "dubious" histological results. Also, between macroscopically "good" and "excellent" samples and histological results that were adequate/diagnostic. And that statistic concordance ( $k = 0.386$ ) was of moderate magnitude according to the "Landis and Koch" classification.

### Discussion

Macroscopic quantitative evaluation of EBUS-TBNA samples is statistically related to histological results, being the macroscopically "excellent" samples more often (88.2%) representative of lymph node or diagnostic.

From the 202 samples classified as "excellent", 11 were considered "inadequate", and 13 were considered "dubious" by final cytologic diagnosis, totalling 11.8%, somewhat higher than other published studies, where samples considered adequate/satisfactory by ROSE [6–9] resulted in about 5% of inconclusive/indeterminate samples by final cytologic diagnosis. This probably means that the histological material was made by cellular "contaminants", acquired when the needle passed through the tracheal wall en route to the targeted lesion, extensive necrosis, blood or insufficient lymphoid or neoplastic cells, therefore not meeting the histological criteria for adequate/positive.

Moreover, when samples were classified as "insufficient" or "sufficient" in our study, 88.9% and 59.1%, were considered "inadequate" or "dubious" by cytology respectively. These percentages are very satisfactory since in a study using ROSE [6], 50.6% of samples classified

**Table 6** Macroscopic evaluation versus histologic evaluation cross tabulation

		Histological classification			Total
		Inadequate	Dubious	Adequate/diagnostic	
Macroscopic evaluation	Insufficient	8 (88.9%)	0 (0.0%)	1 (11.1%)	<b>9 (100.0%)</b>
	Sufficient	7 (31.8%)	6 (27.3%)	9 (40.9%)	<b>22 (100.0%)</b>
	Good	11 (20.0%)	2 (3.6%)	42 (76.4%)	<b>55 (100.0%)</b>
	Excellent	11 (5.4%)	13 (6.4%)	178 (88.1%)	<b>202 (100.0%)</b>
Total		<b>37 (12.8%)</b>	<b>21 (7.3%)</b>	<b>230 (79.9%)</b>	<b>288 (100.0%)</b>

as inadequate remained inadequate on final cytologic diagnosis.

This study had some limitations. It was conducted in a single centre, and the macroscopic evaluation might be considered too subjective. Also, because ROSE is not available in our institution, there was no direct comparison/agreement between ROSE and our macroscopic evaluation of the same sample.

On the other hand, the proposed macroscopic classification in this study had positive statistical results, not too far from ROSE, and does not require any further economic or human resources, that is, a pathologist readily available at the time of the procedure, which most institutions do not dispose. It is a practical, easy, cheap, fast and on-site classification that provides immediate feedback to the operator, potentially shortening the duration of the procedure and/or ensuring a higher percentage of samples with a diagnosis.

## Conclusions

Although it is a subjective way of quantifying samples, authors consider this findings of high clinical importance since this represents a fast, cheap and easy method that evaluates the adequacy and representativeness of the material obtained for final interpretation by cytology in the absence of ROSE. This immediate macroscopic evaluation should influence the procedure, namely, obtaining additional passes trying to achieve an “excellent” sample and thereby potentially lowering nondiagnostic sampling.

## Abbreviations

EBUS: Endobronchial ultrasound; EBUS-TBNA: Endobronchial ultrasound-guided transbronchial needle aspiration; IASLC: The International Association for the Study of Lung Cancer; ROSE: Rapid on-site cytology examination.

## Authors' contributions

MIM conceived this article. JNC contributed for the data analysis. MdS and PM are the pneumologists that acquired the EBUS-TBNA samples and classified them macroscopically. ALF and LB revised the article critically for important intellectual content. All the authors contributed to the interpretation of the results and have read and approved the manuscript.

## Funding

Not applicable.

## Availability of data and materials

We are available to provide the study data.

## Declarations

### Ethics approval and consent to participate

This study was approved by the ethical committee of Portuguese Institute of Oncology of Coimbra. Reference number not available.

### Consent for publication

Not applicable — identifying images or other personal or clinical details of participants was not presented so that anonymity is not compromised.

### Competing interests

The authors declare that they have no competing interests.

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