The value of rapid on-site evaluation during endobronchial ultrasound-guided transbronchial needle aspiration in the diagnosis of mediastinal lesions

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Introduction Rapid on-site evaluation (ROSE) is a technique used for immediate interpretation of transbronchial aspirates; there is debate as regards the contribution of ROSE to the diagnostic or staging process in patients with lung cancer undergoing endobronchial ultrasound-guided transbronchial needle aspiration (EBUS-TBNA).

Aim of the study The aim of this study was to evaluate the role of ROSE during EBUS-guided TBNA in the diagnosis of the nature of mediastinal/hilar lesions detected using thoracic computed tomography regardless of whether or not there is a known lung malignancy.

Patients and methods All patients with hilar/mediastinal lymph nodes having short axis of at least 1 cm on new thoracic computed tomography scan were included. The target lymph node was examined using real-time EBUS B-mode, and then TBNA was performed by inserting a dedicated 22-G needle through the working channel of the bronchoscopy. The samples were examined using the ROSE technique and then compared with the results of the final pathologic diagnosis.

Results The current study was conducted on 47 patients (29 male and 18 female) with a age (mean±SD) of 56±15.4 years. EBUS-TBNA was performed for 129 mediastinal lesions in different stations. Among them, 77 of them were diagnosed as

malignant initially on performing ROSE. After final histopathological and immunological examination, 74 lesions were proved to be malignant, whereas three lesions turned out to be benign. 52 lesions were diagnosed as benign on ROSE, whereas four of them were proved to be malignant on the final diagnosis, giving ROSE a specificity of 94.12%, sensitivity of 94.87%, and diagnostic accuracy of 94.57%.

Conclusion ROSE has add-on advantages to EBUS-TBNA in many aspects, increasing diagnostic accuracy of EBUS-TBNA, increasing safety, and providing sufficient samples for subsequent immunocytochemical and molecular analysis.
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Introduction

Sampling is essential in the diagnosis of malignant mediastinal or hilar lymph nodes (LNs) and lung cancer staging. Early diagnosis with the least-invasive method and acquisition of sufficient tissue are very important. However, it may be difficult to acquire accurate information using conventional modalities such as bronchoscopy and radiology [1]. American College of Chest Physicians and the European Society of Thoracic Surgeons recommend staging by means of tissue diagnosis from suspicious LNs [2,3].

Endobronchial ultrasound-guided transbronchial needle aspiration (EBUS-TBNA), developed in 2004, is considered as a minimally invasive procedure in the diagnosis and staging of lung cancer with a sensitivity ranging from 88 to 93% [4]. Its role expands to include the diagnosis of mediastinal lymphadenopathy and other mediastinal lesions [5].

Rapid on-site evaluation (ROSE) is a technique used for immediate interpretation of transbronchial aspirates. It has a positive impact on the diagnostic yield of TBNA

and to avoid futile additional biopsies [6]. The role of ROSE during EBUS-TBNA is controversial because EBUS-TBNA allows for better real-time visualization and sampling of the lesions. Hence, it may limit the beneficial role of ROSE during EBUS-TBNA [7].

The aim of the present study was to evaluate the role of ROSE during EBUS-TBNA in the diagnosis of the nature of mediastinal/hilar lesions detected using thoracic computed tomography (CT) regardless of whether or not there is known lung malignancy.

Patients and methods
Patients
This prospective study was performed during the period from November 2015 to October 2016 at Tanta University Hospital and Kobry-ElKoba Military Hospitals. Any patient with hilar/mediastinal lymph

nodes (having short axis ≥1 cm on new thoracic CT scan) or with hilar and/or mediastinal lymph nodes positive on PET/CT scan without considering the diameter was included in this study. However, any patient with cardiovascular instability, bleeding diathesis (international normalized ratio<1.3 or platelet count of <50 000/mm^3), presence of endobronchial lesion, presence of definitive distant metastasis, or former chemotherapy or radiotherapy was excluded. All patients were subjected to full clinical examination, new CT examination with contrast (EBUS-TBNA was performed within maximum 5 days of CT examination), and coagulation profile before the procedure. All patients signed an informed written consent form and the study protocol was approved from research ethics committee of Faculty of Medicine, Tanta University. The study protocol was registered on clinicaltrial.gov with the number NCT02690610.

Study procedure

Patients were examined in the supine position under local anesthesia with 2% lidocaine sprayed to the larynx and moderate sedation (8.56±2.31 mg midazolam). First, dedicated fiberoptic bronchoscope (Pentax EPK-i5000; Pentax, Tokyo, Japan) was inserted orally to examine all patients' airways. Thereafter, convex probe-EBUS (HI VISION Avius; Hitachi Company, Tokyo, Japan) was brought in contact with all targeted LNs, as revised using CT scan. Color Doppler ultrasound was used to avoid puncture of surrounding vascular lesions.

LNs were identified according to Mountain's regional LN classification system [6]. The target LN was examined using real-time EBUS B-mode to assess its shape, echogenicity, edge definition, and diameter.

TBNA was performed using a dedicated 22-G needle (ECHO-HD, 22-EBUS P, Echotip, Ultra; Cook Ireland Ltd, Limerick, Ireland) inserted through the working channel of the bronchoscopy. Two-to-four attempts of aspiration under negative pressure by moving the needle gently to and fro inside the targeted lesion were made each time of aspiration to increase the core of cells aspirated. The number of TBNA per each station was calculated. Lymph node stations (2, 4, 7, 10, and 11) were accessible and could be evaluated. N3 nodes were punctured first followed by N2 and N1 to avoid implantation of cancer cells and false-positive results.

Specimen management

This study was performed using the ROSE technique. The material aspirated was smeared on several glass slides. The slides were alcohol-dried and processed using Diff-Quick stain for immediate examination. The decision of further sampling or to terminate the process depended on ROSE results.

Thereafter, cell blocks were prepared from clotted material in the syringes used for fine-needle aspiration cytology. The aspirate was rinsed in 10 ml of 50% ethanol in a specimen container. The entire material was centrifuged in a 10 ml disposable centrifuge tube. The deposit was fixed in 100% ethanol and one part of 40% formaldehyde. The cell blocks were embedded in paraffin and sectioned at 4 μm thickness. Routine H&E (Harris H&E, Tucson, AZ, USA) staining was used on all cell block sections. According to the need, a range of monoclonal antibodies, chromogranin, LCA, TTF1, CD3, CD20, CD15, pancytokeratin, CK7, CK20, and MUM1, were used. According to the manufacturer's instructions for the kits (Dako, Glostrup, Denmark), it was applied in patients requiring identification or phenotyping of tumor cells using the streptavidin biotin method.

Diagnosis

The specimens were either positive (a definitive diagnosis of malignancy or the presence of caseating or noncaseating granuloma), negative (benign lymphocytes were present (≥40 lymphocytes in a high-power field)) [8], suspicious (only dysplastic cells), or nondiagnostic (inadequate specimen containing blood, mucus, or respiratory epithelial cells). For positive and suspicious cases cell blocks combined with immunophenotyping were used to confirm or exclude the diagnosis of malignancy and for further subclassification of malignant cases. Radiologic follow-up was carried out on the outcome of the LNs for at least 6 months. On follow-up, LNs that persisted in size, diminished, or resolved were confirmed to be benign. The results of ROSE were compared with the results of the final pathologic diagnosis (the gold standard). Two pathologists independently interpreted the results.

Statistical analysis

All data were presented as a mean±SD. Sensitivity, specificity, positive predictive value, negative predictive value, and diagnostic accuracy rate were calculated from the standard definitions used for malignancy diagnosis. Statistical analyses were performed by using SPSS software (version 17.0; SPSS Inc., Chicago, Illinois, USA). Differences were considered statistically significant when P was less than 0.05.

Results

The current study was conducted on 47 patients (29 male and 18 female) with a age (mean±SD) of 56±15.4 years.

EBUS-TBNA was performed for 129 mediastinal lesions in different stations as follows: 13 located in the upper paratracheal stations: two right and two left, 32 located in the lower paratracheal stations: four right and four left, 39 located in the subcarinal station 7, 27 located in the hilar group stations 10 right and 10 left, and 18 located in the interlobar group stations 11 superior, 11 inferior, and 11 left (Table 1 and Fig. 1).

The operating time was 31±6.4 min with no bronchoscopy or TBNA-related complications. All patients tolerated the procedure with no recorded complications. Among the 129 lesions, 77 of them were diagnosed as malignant initially on performing ROSE. After final histopathological and immuno-logical examination on cell blocks, 74 lesions were proved to be malignant, whereas three lesions were benign (two reactive lymphadenitis and one granulomatous lymphadenitis). Overall, 52 lesions were

diagnosed as benign on ROSE, whereas four of them were proved to be malignant on the final diagnosis. The four cases were diagnosed finally as Sclerosing Hodgkin lymphoma (two cases) and metastatic carcinoma (two cases). Hence, the specificity of ROSE was 94.12%, sensitivity was 94.87%, positive predictive value was 96.1%, negative predictive value was 92.31%, and accuracy was 94.57%.

Malignancy was diagnosed in 78 lesions (13 adenocarcinomas, 10 squamous cell carcinomas, 14 nonsmall-cell lung cancer not otherwise specified, 14 small-cell lung cancer, 10 non-Hodgkin lymphomas, seven Hodgkin lymphomas, and 10 other malignant tumors as neuroendocrine tumors, metastasis from breast cancer, and metastasis from colorectal tumors). Benign condition was found in 51 lesions (13 sarcoidosis, 13 tuberculosis, nine reactive hyperplasia, five nonspecific infections, seven infectious mononucleosis, and four silicosis) (Table 2, Figs. 2 and 3).

Number of passes required for diagnosis in malignant LNs was 1.73±0.65, which was significantly lower than those in benign LNs, which was 2.32±1.21 (t=4.15 and P=0.001).

Discussion

Our study aimed to demonstrate the add-on benefit of ROSE to EBUS-TBNA in the simplicity and efficacy of the diagnosis of the mediastinal lesions. From our results, on performing ROSE we initially found 77 malignant lesions. After final histopathological and immunological examination, 74 lesions were proved to be malignant, whereas three lesions were benign. Overall, 52 lesions were diagnosed as benign on ROSE, whereas four of them were proved to be malignant on the final diagnosis, giving ROSE a

Table 1 Patients' demographics and characteristics

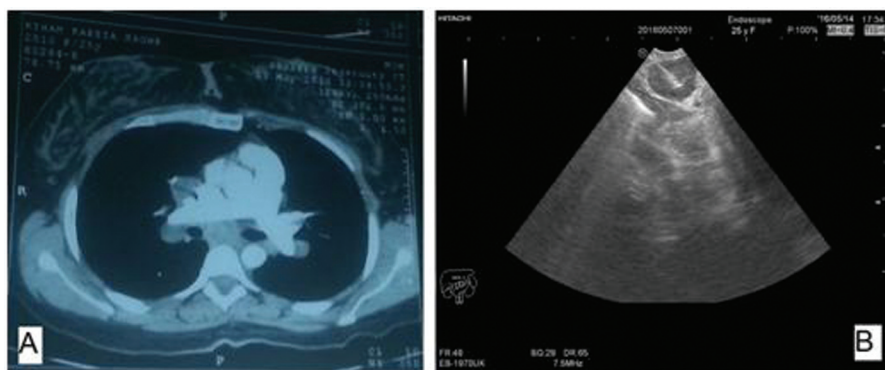|	
Age (mean±SD) (years)	56±15.4
Sex [n (%)]	
Male	29 (61.7)
Female	18 (38.3)
LN stations [n (%)]	
2R and 2L	13 (10.08)
4R and 4L	32 (24.81)
Station 7	39 (30.23)
10R and 10L	27 (20.93)
11s, 11i, and 11L	18 (13.95)
Number of passes	
Benign lesions (mean±SD)	2.32±1.21
Malignant lesions (mean±SD)	1.73±0.65
t	4.15
P	0.001*

Nodal stations are classified according to the classification of American Thoracic Society. 2, upper paratracheal; 4, lower paratracheal; 7, subcarinal; 10, hilar; 11, interlobar. i, Inferior; L, left; LN, lymph node; R, right; s, superior. *Significant.

Figure 1

A 25 year female patient complained from dyspnea and nonproductive cough. (a) Computed tomography scan shows a large subcarinal lymph node (station 7). (b) Endobronchial ultrasound-guided transbronchial needle aspiration was done, diagnosed as lymphoblastic non-Hodgkin lymphoma

Table 2 Final diagnosis of different mediastinal lesions

| Malignant lesions (N=78)	Benign lesions (N=51)
13 Adenocarcinomas	13 Sarcoidosis
10 Squamous cell carcinomas	13 Tuberculosis
14 Nonsmall-cell lung cancer not. otherwise specified	Nine reactive hyperplasia
14 Small-cell lung cancer	Five nonspecific infections
10 Non-Hodgkin lymphoma	Seven infectious mononucleosis
Seven Hodgkin lymphoma	Four silicosis
10 Other malignant tumors such as neuroendocrine tumors, metastasis from breast cancer, and metastasis from colorectal tumors	

Figure 2
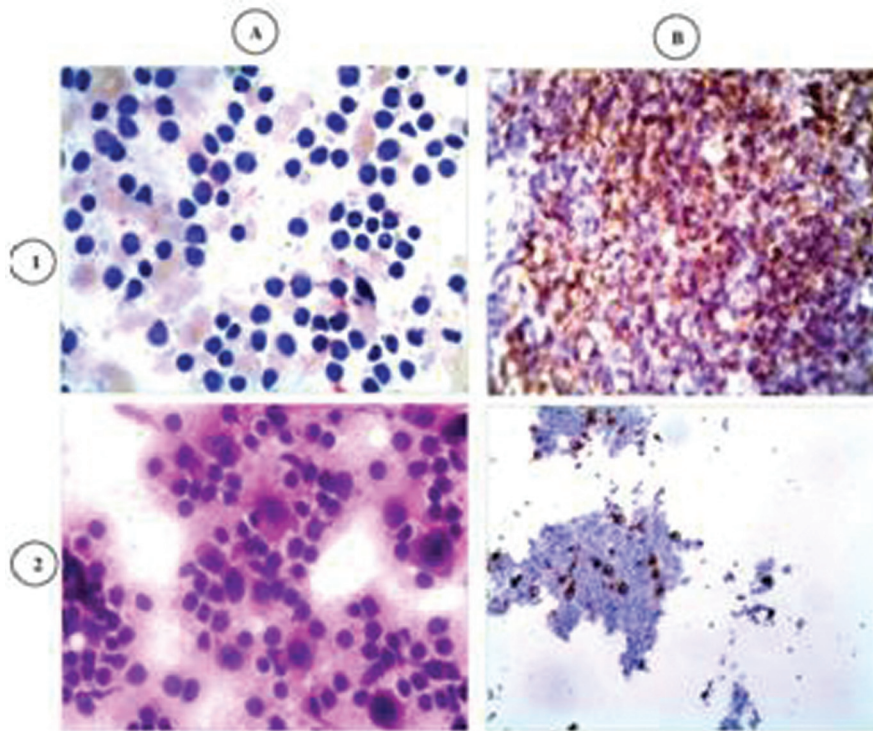
(1a) Fine-needle aspiration cytology (FNAC) showing large atypical lymphoid cells suspicious for large cell lymphoma (Diff Quick ×400). (1b) Cell block showing positive CD20 immunostaining of neoplastic large cells (streptavidin biotin ×400). (2a) FNAC showing atypical cells with eosinophilic nucleoli suspicious for Hodgkin lymphoma (Diff Quick ×400). (2b) Cell block showing positive CD15 immunostaining of R-S cells (streptavidin biotin ×100)

specificity of 94.12%, sensitivity of 94.87%, and diagnostic accuracy of 94.57%. The misdiagnosis of the three cases as malignant (two reactive lymphadenitis and one granulomatous lymphadenitis on final diagnosis) can be attributed to the interpretation of follicular center cells as immature lymphocytes in two cases and hardly detected epitheloid cells in the other case on cytomorphology. However, the misdiagnosis of the four cases as benign (two nodular sclerosing Hodgkin lymphomas and two metastatic carcinomas on final diagnosis) may be attributed to the nature of the lesion and may limit diagnostic cellular yield (sclerosis, desmoplasia, and scanty diagnostic cells in nonspecific background). These results are in accordance with the previous reports [6,9]. Guo et al. [9] reported that the concordance between on-site findings and final pathologic diagnoses in their study was very high (218/221, 98.6%).

Second, the number of passes required for the diagnosis of mediastinal lesions using ROSE was less than that without its assistance [9,10]. In our study, the number of passes for the diagnosis of malignant lymph nodes was 1.73±0.65, which was significantly lower than those of benign lymph nodes, which was 2.32±1.21 (t=4.15 and P=0.001) with an operating time of 31±6.4 min, and there were no bronchoscopy or TBNA-related complications. Oki et al. [10] documented in their study that ROSE can reduce the number of extrasampling as mean puncture number was significantly lower in the ROSE group (2.2 vs. 3.1 punctures, P<0.001).

Figure 3
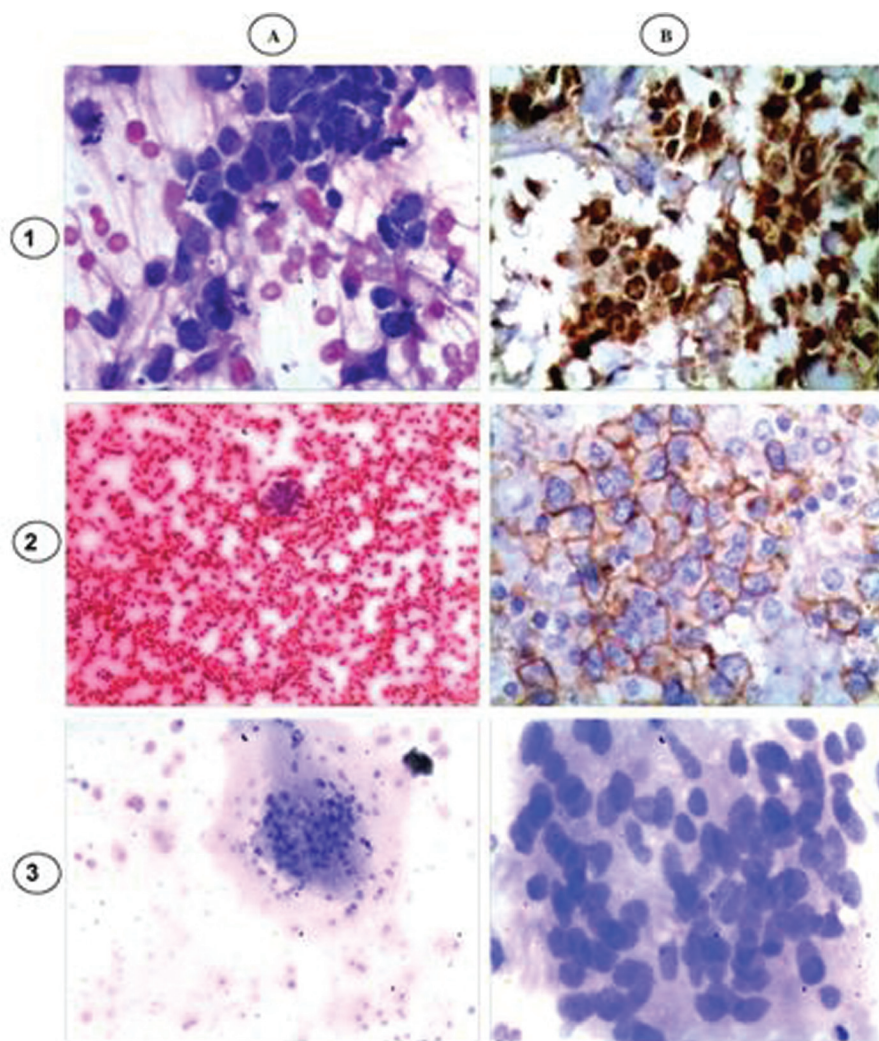
(1a) Fine-needle aspiration cytology (FNAC) small atypical cells suggesting oat cell carcinoma (Diff Quick ×400). (1b) Cell block same case positive chromogranin immunostaining (streptavidin biotin ×400). (2a) FNAC groups of malignant epithelial cells among mature lymphocytes suspicious for metastatic carcinoma (Diff Quick ×400). (2b) Cell block same case positive cytokeratin immunostaining (streptavidin biotin ×400). (3a) FNAC epithelioid cells admixed with mature lymphocytes suggesting granulomatous lymphadenitis (Diff Quick ×400). (3b) Cell block same case groups of epithelioid cells (H&E ×400)

Third, ROSE could reduce the percentage of inadequate specimens and reduce the false-negative results to a certain degree [11–13] as it can differentiate between inconclusive samples due to highly suspicious atypical cells from nondiagnostic samples due to insufficient or necrotizing tissue increasing the diagnostic accuracy. Guo *et al.* [9] agreed as regards this advantage of ROSE and reported in their study that the reduction of the suspicious results in the ROSE group (8.7%) versus the non-ROSE group (14.6%) ($P=0.038$) and the reduction of suspicious ROSE interpretations resulted in an improvement of the diagnostic yield of pathologic samples (90.5 vs. 81.2%, $P=0.003$). Therefore, they could modify the subsequent sampling strategy in real-time, which was performed to retrieve a better sample. Moreover, they encountered suspicious specimens contaminated from potentially involved

bronchial mucus; therefore, they could exclude patients who had intrabronchial abnormalities so as to 'rationalize' a positive diagnosis.

Moreover, ROSE can offer adequate sample, especially when using 22-G TBNA needle, not only for cytological diagnosis but also for immunocytochemical and molecular analysis that will guide in identifying patients who will respond better to certain types of chemotherapeutic agents (target therapy). van Eijk *et al.* [14] documented in their study on 43 patients with nonsmall-cell lung cancer who underwent analysis of some markers (KRAS, EGFR, BRAF, and PIK 3CA mutation) in their specimens acquired by means of EBUS-TBNA that there was complete concordance between the results obtained by the analysis of tumor markers performed on the cytological specimens and

those obtained on surgical histological specimens. Our false-positive and negative cases in this study could be attributed to the use of cell blocks rather than surgical specimens for histopathological and immunological diagnosis in the current study.

In our study, we could diagnose a wide variety of cases both benign and malignant (primary or metastatic). This adds to the volume of data about the beneficial effect of ROSE during EBUS-TBNA in all mediastinal lesions regardless of its size or echogenecity.

The limitation of our study is that all cases were evaluated by the same attending cytologist and this may give a chance for possible misdiagnosis as this procedure is mainly operator and cytologist dependent. To minimize this potential bias, the sample was then consecutively pathologically examined by another cytologist not informed about the provisional diagnosis for confirmation.

Conclusion

ROSE has add-on advantages to EBUS-guided TBNA in many aspects; increasing diagnostic accuracy of TBNA by excluding suspicious or nondiagnostic specimens, increasing safety by decreasing the number of punctures, and providing sufficient samples for subsequent immunocytochemical and molecular analysis needed for meticulous pathological diagnosis and consequential treatment options.

Conflicts of interest
There are no conflicts of interest.

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