Diagnostic yield of rapid on-site evaluation transbronchial needle aspiration versus conventional transbronchial needle aspiration: a single center experience

Carmine Guarino¹, Mariano Mollica², Cristiano Cesaro¹, Adriano Costigliola², Maria Carolina Michelli³, Nadia Corcione², Giuseppe La Cerra¹, Carmelindo Tranfa², Salvatore Cirillo², Riccardo Smeraglio⁴, Rosario Salvi⁵

¹Bronchology Unit, Monaldi Hospital, A.O. dei Colli, Naples; ²Department of Translational Medical Sciences, University of Campania “L. Vanvitelli”, Monaldi Hospital, Naples; ³Pathology Unit, Monaldi Hospital, A.O. dei Colli, Naples; ⁴Department of Public Health, University of Naples Federico II; ⁵Department of Thoracic Surgery, Monaldi Hospital, A.O. dei Colli, Naples, Italy

Abstract

Trans-bronchial needle aspiration allows lymph node sampling in several thoracic conditions; the ability of Rapid On-Site Evaluation (ROSE) to predict the final diagnosis in this setting has not been well characterized. We performed a retrospective study to establish the utility of ROSE in the diagnosis of thoracic diseases with mediastinal lymph node involvement. We retrospectively reviewed 297 patients with hilar-medistinal lymph node enlargement detected at CT scan from January 2013 to April 2016. 201 patients underwent conventional TBNA; in 96 patients, TBNA procedure was performed by on-site presence of a team of pathologists and research morphologists. Lung neoplasms, sarcoidosis, infections and lymphoma were the most common diseases diagnosed with TBNA samples. TBNA simultaneously performed in combination with ROSE produced an increase in percentage of appropriate samples compared to single cTBNA (adequate samples cTBNA vs ROSE-TBNA: 73% vs 81%; p<0.05).

Conclusion: ROSE may serve to reduce procedure time and enhance sample triaging therefore limiting the need for further invasive diagnostic testing.

Introduction

Lung cancer is the leading cause of cancer death worldwide; tobacco smoking and air pollution exposure are mainly implicated as risk factors [1-3]. Despite progresses in diagnostic procedures [4-9] lung cancer diagnosis occurs, in the majority of cases, when surgical approach is no longer feasible [10-12]. Lung cancer treatment has been renovated by target therapy and immune therapy [13-17] as a result of advances in understanding molecular basis of cancerogenesis [18-30]. This imply the need of adequate sampling procedures.

Trans Bronchial Needle Aspiration (TBNA) is a minimally invasive bronchoscopic technique which allows cytological, histological, or microbiological sampling of lesions within the airway wall, the lung parenchyma, and mediastinum adjacent to the tracheo-bronchial structures. Fine needles (usually 19 and 22 gauge) are introduced through the wall of the airway into a lymph node in order to obtain a specimen for cytological, histological or microbiological analysis. Hilar-medistinal lymph node enlargement represent a major diagnostic challenge for both neoplastic and benign thoracic diseases [31-33]. TBNA is widely used procedure compared to more invasive mediastinoscopy and video-thorac-
coscopy for tissue specimen collection in lung cancer diagnosis and staging [34].

The diagnostic yield of TBNA may be influenced by several factors, including the operator experience, type of needle used, size and position of the lesion and prevalence of disease in the study population. Awareness of these factors, as highlighted by Bonifazi and colleagues [35], allows appropriate procedure planning which positively affects diagnostic accuracy.

The Rapid On-Site Evaluation (ROSE) is the intra-operative cytological evaluation of samples obtained by fine needle aspiration procedures, which has been shown to increase the diagnostic yield of samples [36]. Even in absence of specific guidelines, a classification of the cytological samples obtained using TBNA has been proposed (Table 1). However, no general consensus on the definition of adequacy of trans-bronchial specimen examined with ROSE is so far available: some authors consider a sample adequate even with absence or low value of lymphocytes with presence of bronchial epithelial cells; other authors consider samples inadequate with all withdrawals (C1, C3, C4) which do not allow a definitive diagnosis. Differing results have been reported in regard of the use of ROSE. In studies ranging from 1990 to 2010, which was mostly observational and not randomized, an increase of adequacy and/or diagnostic sensitivity of trans-bronchial samples assessed by the cytologist was reported. The randomized trials from 2011 to date, conducted by Trisolini [37] have shown that the ROSE was able to reduce the number of withdrawals for the diagnosis, without implications regarding the diagnostic yield, reducing the time of execution of the examination, and ultimately reducing the percentage of complications related to endoscopic maneuver (respiratory failure, bronchospasm, cardiac arrhythmias and hemodynamic instability, bleeding, fever). Baram and colleagues [38], have shown that a pre-procedural planning is able to predict the conditions in which the ROSE may have a better diagnostic yield. In fact, the ROSE may avoid additional biopsies to the patient where there is adequacy of the sample even in presence of multiple targets. Inadequate tissue sampling due to false positivity procedure represents the major risk for unsuccessful intra-operative cytological assessment: a close collaboration between endoscopist and pathologist may reduce this risk. As reported by Shannon et al. [39], the cTBNA + ROSE and EBUS-TBNA + ROSE methods seem to be overlapping in terms of diagnostic yield.

The costs related to the presence of the pathologist in endoscopic room for ROSE was investigated by Bonifazi et al. [40] in comparison to the procedure performed by clinician trained by a short educational program of pulmonary pathology: the results indicate a procedural costs reduction. However, these studies were conducted in a single center with high-experience in cTBNA and EBUS-TBNA techniques, with established collaboration with pathologists. The impact of ROSE on diagnostic yield in different setting has not been investigated.

Although the EBUS-TBNA, according to recent observations, is able to guarantee a greater diagnostic yield compared to cTBNA, there is still need to explore the effectiveness cTBNA as EBUS is not available in all centers due to high costs of the equipment and involvement of a multidisciplinary team, including anesthetist. The aim of this study is to determine the impact of extemporaneous cytological evaluation (ROSE), in terms of adequacy of the sampling and diagnostic yield of TBNA on hilar or mediastinal lymph nodes detected by chest CT scan with contrast.

**Table 1. TBNA/ROSE specimens classification.**

| classification | Definition |
|----------------|------------|
| C1             | sample not adequate or not evaluable |
| C2             | benign outcome (normal cells, inflammatory disease) |
| C3             | unclear outcome (cells with characteristics not completely attributable to benign disease) |
| C4             | unclear outcome, probably malignant (cells with morphological atypia, in absence of specific features of malignancy) |
| C5             | malignant outcome |

**Table 2. Main features in the two groups under examination.**

|                      | TBNA group (n=105) | TBNA + Rose group (n=96) |
|----------------------|--------------------|--------------------------|
| Age                  | 63 (55-71)         | 65 (53-69)               |
| Male                 | 41                 | 39                       |
| Cardiovascular comorbidity | 30              | 37                       |
| Respiratory failure | 5                  | 7                        |
| Sampled lymph nodes  | 2 (1-3)            | 3 (1-4)                  |

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procedure, including blood pressure, heart rate, respiratory rate, and oxygen saturation. The location of the bronchus leading to the lesion was planned on the basis of CT features. For the ROSE group, the materials from forceps biopsy imprint cytology were smeared onto glass slides. The glass slides were rapidly air-dried and stained immediately with a modified rapid Giemsa stain (Diff-Quik stain). Microscopic examination of the slide preparation with Diff-Quik stain was performed within the bronchoscopy suite by two experienced cytotechnologists. In this manner, ROSE diagnosis was immediately communicated to the bronchoscopists. A cytotechnologist determined specimen adequacy.

Our diagnostic criterion for adequacy is the presence of cancer cells or cells suspicious for malignancy. The samples were classified as C1 to C5 according to the classification reported above. We considered a withdrawal as inadequate in presence of a small number of lymphocytes and prevalence of tracheobronchial epithelium cells. The sample was defined diagnostic if was within the C2 and C5 categories. If ROSE is positive, we will limit the sampling at this site to ensure the reliability of the results, reduce the risk of injury and bleeding. If ROSE is negative, we will repeat the biopsy. If all samples are negative according to the imaging prejudgment, and malignant disease is highly suspected clinically, we will recommend that patients undergo other tests, such as percutaneous puncture and thoracosopic biopsy, in order to reduce the time until diagnosis. In each case, the remaining materials (ROSE group) or all specimens (non-ROSE group) were placed in 10% formaldehyde and embedded in paraffin for routine histologic examination on hematoxylin and eosin staining. ROSE diagnoses were compared with the final pathologic diagnoses. The final diagnosis was based on the analysis of routine histologic pathology. The term malignant was defined as the presence of cancer cells or cells suspicious for malignancy. The samples were categorized as positive for malignancy or negative for malignancy, based on the results of ROSE. If flexible bronchoscopy produced a nonspecific diagnosis of malignancy, patients were referred for repeat flexible or rigid bronchoscopy, video-assisted thoracic surgery, or other surgical procedures. Data obtained were processed using the chi-square test.

Results

Of the 201 samples taken with conventional TBNA technique without on-site cytological evaluation, 55 samples were inadequate and 146 adequate (percentage of adequacy: 73%). Of the 96 samples taken with ROSE + TBNA technique, 11 samples were inadequate and 85 were adequate (percentage of adequacy: 86.3%) with *p* < 0.05 (Figure 1A). Figure 2 reports cytology assessment of lymph node specimens collected with ROSE-TBNA procedure. TBNA simultaneously performed in combination with ROSE produce an increase of percentage of appropriate samples compared to single cTBNA (*p* < 0.05). In addition to the accuracy for histological diagnosis, ROSE in our study as exhibited a relevant favorable impact on adequacy of sample for molecular profiling. Of the 146 adequate cTBNA samples, a conclusive diagnosis (C2-C5) was achieved in 104 patients. The remaining 42 samples resulted in a non-definitive diagnosis. Among 85 adequate samples made with TBNA + ROSE technique, 70 patients obtained a conclusive diagnosis; 15 samples were not diagnostic results (C3-C4) (percentage of diagnostic success: 81%) with *p* < 0.05. The most common diseases diagnosed which ROSE were primary tumor of the lung, sarcoidosis, TBC, lymphoma (Figure 1B). There were no false positive cases in the ROSE group. A second investigation level such as mediastinoscopy or video-assisted thoracoscopy (VATS) was required in patients with non-conclusive diagnosis. In addition to the impact of ROSE on diagnostic yield of TBNA sampling we have also explored the relevance of this methods on adequacy of samples for molecular profiling. We have considered adequate for a correct molecular profiling a samples exhibited at least 20% of tumor cell nuclei. According to this cut-off, 97% of samples assessed with ROSE were adequate for molecular profiling.

Discussion

In this study, comparing a group of patients undergoing cTBNA with a group of patients undergoing TBNA + ROSE, we have demonstrated an increase in adequacy of fine needle aspirations and increased diagnostic yield in the group of patients where ROSE was performed. In addition, our observations highlight the importance of correct pre-operative planning in optimizing the endoscopic procedures and sampling collection in terms of assessment of number of lesions to be sampled, lymph node location, size of the lesions, selection needle type and sequence of sampling.

Conventional TBNA has been used for thirty years as a valuable tool in the diagnosis of hilar-mediastinal lymph nodes enlargement. However, according to the recent reports of the literature, the cTBNA and EBUS-TBNA guarantee a similar diagnostic yield when
used by experienced endoscopists. The role of ROSE of the samples obtained by trans-bronchial needle aspiration remains controversial. In a previous study, Shannon and colleagues [39] showed that the EBUS-TBNA have diagnostic performance comparable to TBNA + ROSE. In the present study, we have compared a group of patients undergoing TBNA with a group of patients undergoing cTBNA + ROSE; the results documented an increase in adequacy of fine needle aspirations and increased diagnostic yield in the ROSE group. Also, in our experience the correct pre-operative planning may optimize the procedure through the evaluation of several factors (number of lesions, lymph node location, size of the lesions, the needle type). The high cost EBUS still represents a limit to the wide spread of this technique. In this scenario, the ROSE can limit sampling bias, maximizing the diagnostic yield; the reduction of execution time of the procedure related to the ROSE also makes this technique a valid tool regarding patient outcomes. A multidisciplinary approach involving pulmonologist and pathologist during bronchial endoscopic procedures could therefore guarantee an accurate diagnosis even in absence of endoscopic ultrasound system.

Conclusions

The ROSE may optimize cTBNA procedure allowing a correct diagnosis in most of patients. The advantages of using this technique include reduction in the number of biopsies and average time and complications of the diagnostic procedure; also, the reduced use of sedative is preferable for the patient. In light of the growing need to obtain adequate tissue sampling for lung cancer diagnosis, the ROSE procedure can act, in absence of the EBUS, as “navigator” for the endoscopist, minimizing the risk of non-diagnostic procedure. In the era of precision medicine, the ROSE could be a relevant diagnostic tool to achieve accurate tissue sampling even for molecular biology investigations.

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