Rapid on-site evaluation: what a microscope will add to the bronchoscopy unit? a concise review

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Rapid on-site evaluation (ROSE) of samples obtained by transbronchial needle aspiration during flexible bronchoscopy or endobronchial ultrasound has been practised for more than two decades. Earlier studies evaluating its role have reported a magical impact on improving the diagnostic yield and the adequacy of samples produced by transbronchial needle aspiration. Subsequent studies with more rigorous methodologies failed to find a significant increase in sensitivity with ROSE but consistently demonstrated a trend toward performing shorter procedures with fewer complications when ROSE is utilized. There are new exciting fronts for ROSE, such as using it to direct molecular testing for lung cancer. In the future, we expect more centers to apply ROSE, now that pulmonologists have succeeded in doing so and telecytopathology has become reality.

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Introduction

In the era of modern medicine and development of sophisticated diagnostic machines that are less invasive and – as a consequence – acquire smaller samples, fine-needle aspiration (FNA) has become a well-established procedure that is commonly used for investigating lesions at many anatomical locations. It is regarded as safe and accurate and has a low complication rate.

Rapid on-site evaluation (ROSE) of cytological materials obtained using FNA procedures has been used for some time for evaluating lesions located in different organs/structures in the body with the aim of fine-tuning the sampling procedure [1].

The concept of FNA was introduced in flexible bronchoscopy in 1983 by the innovation of transbronchial needle aspiration (TBNA) with the aim of sampling abnormal structures beyond the airways (the mediastinum) [2]. It has become a prominent sampling tool for a variety of malignant, infectious, and granulomatous lesions, and in the setting of nonsurgical staging of lung cancer TBNA has been shown to decrease the need for diagnostic thoracic surgery [3].

Using ROSE during TBNA was first studied by Davenport [4] who was the first to publish about the subject in a major journal. The positive results in terms of improved diagnostic yield have encouraged large centers to incorporate ROSE in their bronchoscopy units. More studies have later looked into the role of ROSE during TBNA.

The addition of real-time ultrasound guidance to the needle during TBNA [called endobronchial ultrasound (EBUS)] was an immense technological breakthrough that has dramatically refined the process of TBNA and has allowed both examination and sampling of very small lesions [5]. The 'blind' procedure was called conventional transbronchial needle aspiration (cTBNA) henceforth to differentiate it from EBUS-guided TBNA. The ultrasound technology did not alienate ROSE. On the contrary, it is now a mark of excellence to have an EBUS machine in addition to the capability to perform ROSE during TBNA.

This review aims at examining the exact role of ROSE during TBNA, whether conventional or EBUS guided, and to point out the added value, if any, in improving diagnostic yield and decreasing complications of endoscopic procedures.

Materials and methods

A search on Medline was performed from 1990 to April 2016 with the following keywords: 'TBNA'; 'ROSE'; and 'on-site cytology'. Entries that were not in English or involved case series with less than 20 patients were excluded. In total; 48 studies could be identified. After examining the titles/abstracts; 21 studies were excluded; and full texts of the

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remaining articles [6] were retrieved for evaluation [4,6–31]. Data on the study design; diagnostic yield; complication rate; and number of patients in these studies are shown in Table 1.

Rapid on-site evaluation

The cellular material retrieved from the TBNA is conventionally smeared on a glass slide, directly 'wet-fixed' in 95% ethanol, and then later sent to the cytopathologist who usually uses either the May–Grunwald Giemsa or the Papanicolaou method to stain slides [32]. Any of these techniques requires around 5 min of preparation per slide. For the purpose of rapid and timely examination of the aspirated material 'on-site' (in the bronchoscopy unit), cytopathologists have devised a modification for the Giemsa method that allows slide preparation within 30 s. There are various commercial kits available, and the most commonly used one (which is reported in more than half of the cited studies) is the Diff-Quik method [32]. In this method, three aliquots containing different solutions are used. After smearing the TBNA material on the slide, it is left to dry in air and then impregnated in each aliquot for 5–8 s, which can then be examined directly. Images obtained can be used to define the adequacy of the sampled material by showing either malignant cells or at least abundant lymphocytes. Sometimes a provisional diagnosis can also be reached. Figures 1 and 2 show smears highly suggestive of nonsmall and small-cell lung cancer, respectively. A smear composed predominantly of red cells or bronchial cells (Fig. 3) denotes an inadequate sample.

Conventional transbronchial needle aspiration and rapid on-site evaluation

Inspecting the studies in Table 1 will clearly show two distinct eras – the first from inception of the idea in 1990 to 2010 and the latter from 2011 onward. Earlier studies were observational in nature and their results showed improved sensitivity with addition of ROSE to TBNA compared with procedures performed without ROSE [4,7]. Diacon *et al.* [9] did not have a comparative group, but demonstrated that the overall costs are significantly lower by having a cytopathologist on-site, avoiding the need for additional diagnostic procedures once a diagnosis is reached.

Later studies in the 'observational' era were more conservative and critical. Although the results of Chin *et al.* [8] favored ROSE for allowing better diagnostic yield, the authors identified a key problem – the extremely high risk of selection bias. No parameters were set for the allocation of patients into the ROSE or no-ROSE arms, a practice that makes it impossible to rule out that more complex cases were allocated to the ROSE arm or vice versa. The question was made even more relevant when Baram *et al.* [10] failed to find any diagnostic superiority by using ROSE during cTBNA. They confirmed, however, the earlier edge of enabling to conclude the procedure after fewer biopsies. At this point, it was felt that the success rate of cTBNA is influenced by a number of factors besides ROSE, such as size and location of lymph nodes, experience of the examiner, the needle type used, underlying disease, and prevalence of the disease being ascertained [33].

The second era was marked by two randomized-controlled studies that were published almost simultaneously. The first trial aimed at evaluating the usefulness of ROSE in clinically unselected patients with lymphadenopathy at computed tomography [12]. Neither diagnostic yield nor specimen adequacy was significantly different in the two study arms. The possibility to avoid biopsy from additional targets without loss in diagnostic yield was the most important benefit of using ROSE, as it was associated with a significant reduction in the complication rate of bronchoscopy. The other trial (which had fewer patients) reported a similar pattern with effect on diagnostic yield and hinted on a 'trend' toward allowing fewer passes with ROSE [13].

Endobronchial ultrasound-transbronchial needle aspiration and rapid on-site evaluation

Despite seeming intuitive that EBUS guidance should obviate the need for ROSE to confirm the value of the sampled material, in real life, most centers that have the capability for ROSE are the ones that are large enough to have the EBUS technology. ROSE for EBUS-TBNA has had a good share of studies looking at it. Griffin *et al.* [15] were the earliest to study the utility of ROSE during EBUS. The authors retrospectively studied the outcomes of 294 EBUS-TBNAs of which 140 had ROSE performed and unexpectedly reported no remarkable difference in diagnostic yield, the number of sites sampled per patient, or clinical decision making between specimens collected through EBUS-TBNA with or without ROSE. Similar findings were reported from a later study that only observed the outcome of EBUS-TBNA without a comparison group [19].

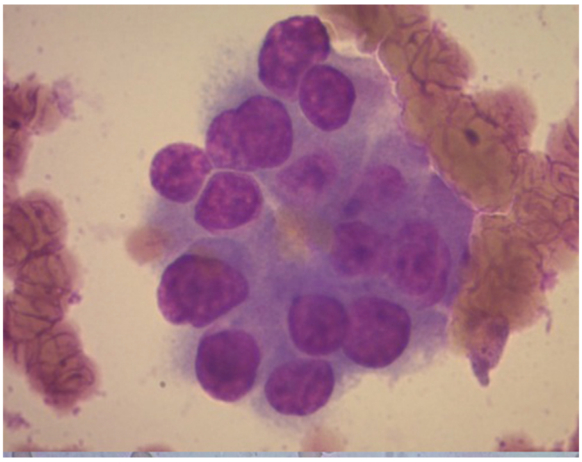
Other studies with observational design that explored the different aspects of ROSE with EBUS came with more positive results. Eapen *et al.* [18] reported the

Table 1 Summary of the studies discussing rapid on-site evaluation for transbronchial needle aspiration

References	Number of patients	Sampling technique	Study design and purpose	Outcome
Davenport [4]	207	cTBNA	Comparative, nonrandomized (73 with ROSE vs. 134 without ROSE)	Improved diagnostic yield (56% for ROSE vs. 31% without ROSE)
Diette *et al.* [7]	204	cTBNA	Comparative, nonrandomized (81 ROSE)	Improved diagnostic yield (80% for ROSE vs. 51% without ROSE)
Chin *et al.* [8]	55	cTBNA	Comparative, nonrandomized (ROSE 55 vs. non-ROSE 35)	Better yield (70 with ROSE vs. 25% without ROSE) Problem with bias possible
Diacon *et al.*[9]	90	cTBNA	Observational	Addition of ROSE allowed the procedure to be terminated early in 64% of cases
Baram *et al.* [10]	44	cTBNA	Comparative, nonrandomized (32 with ROSE vs.12 without ROSE)	No difference in yield. Fewer biopsies needed in the ROSE group.
Cardoso *et al.* [11]	81	EBUS	Comparative, nonrandomized (41 with ROSE vs. 40 without ROSE)	93 vs. 80% sensitivity in favor ROSE
Trisolini *et al.* [12]	168	cTBNA	RCT	No difference in diagnostic yieldLess number of passes and fewer complications with ROSE
Yarmus *et al.* [13]	68	cTBNA	RCT	No difference in diagnostic yieldTrend toward fewer passes with ROSE
Griffin *et al.* [15]	294	EBUS	Retrospective comparative (140 cases with ROSE)	No difference in sensitivity or number of procedures performed
Brundyn *et al.* [14]	48	cTBNA	Safety and yield in SVC	High yield, less need for biopsyNo complication
Plit *et al.* [16]	60	EBUS	Prospective for sarcoidosis. ROSE versus final diagnosis by TBLB	Concordance rate 92%
Nakajima *et al.* [17]	438	EBUS	Retrospective comparative (ROSE vs. final diagnosis)	Concordance rate 94%
Eapen *et al.* [18]	1317	EBUS	Acquire registry. Rate of complication during EBUS	Less complications with ROSE (less need for TBLB)
Joseph *et al.* [19]	170	EBUS	Retrospective observational	ROSE did not impact sensitivity
Bruno *et al.* [20]	120	cTBNA	RCT	Improved sensitivity and less cost
Oki *et al.* [21]	108	EBUS	RCT	Study not powered to detect improvement in sensitivity. Lower need for additional procedures and punctures
Sindhwani *et al.* [22]	40	cTBNA	Observational	ROSE improved yield and helped prevent repeating procedures
Khurana *et al.* [23]	200	EBUS	Comparative, nonrandomized (telecytology vs. conventional ROSE)	Comparative concordance
Bonifazi *et al.* [24]	84	cTBNA	ROSE by pulmonologist vs. cytopathologist	80% agreement
Minami *et al.* [25]	35	EBUS	Role of Bioevaluator with ROSE	Technique is useful to determine adequacy before microscopy
Murakami *et al.* [26]	77	EBUS	Retrospective (Role of ROSE in SCLC cases)	No difference in sensitivityFewer passes and stations with ROSE
Jeffus *et al.* [27]	118	EBUS	RetrospectiveEvaluated the use of structured ROSE approach to define adequacy	Improved sensitivity with structured approach
Trisolini *et al.* [6]	126	EBUS	RCT. Evaluated the suitability of samples for molecular markers	ROSE provided better samples for molecular markers and allowed fewer passes
Mallya *et al.* [28]	77	EBUS	Observational	85% sensitivity
Guo *et al.* [29]	245	EBUS	Retrospective (122 patients with ROSE, 123 without ROSE)	No difference in sensitivityFewer passes in ROSE group
Madan *et al.* [30]	41	cTBNA	Retrospective, observational	Sensitivity 78% with ROSE
Rokadia *et al.* [31]	255	EBUS	Retrospective, observational granulomatous disease	Concordance rate 80% ROSE with final

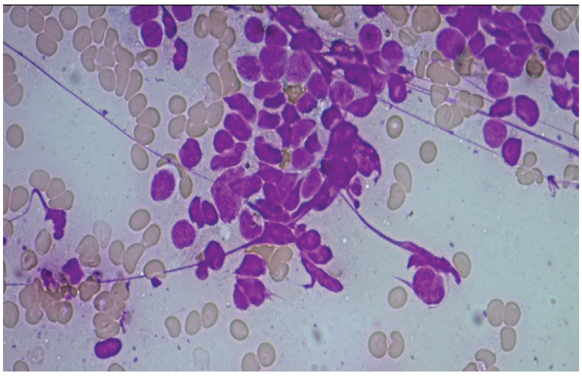
cTBNA, conventional transbronchial needle aspiration; EBUS, endobronchial ultrasound; RCT, randomized-controlled trials; ROSE, rapid on-site evaluation; SCLC, small-cell lung cancer; SVC, superior vena cava; TBLB, transbronchial lung biopsy.

Figure 1



Nest of cells harboring features of malignancy – suggestive of non-small-cell lung cancer. Diff-Quik stain, ×40 magnification.

Figure 2



Nest of cells harboring features of malignancy with scanty cytoplasm – suggestive of small-cell lung cancer. Diff-Quik stain, ×40 magnification.

Figure 3

Bronchial cells by Diff-Quik stain.

findings of the acquire registry created by the American College of Chest Physicians, where they found that the rate of complications was significantly less during EBUS-TBNA when ROSE was used, and they explained that this was mainly due to performing less transbronchial biopsy (TBBX) procedures when ROSE was used. Both Murakami *et al.* [26] (who studied specifically cases that were eventually diagnosed with small-cell lung cancer) and Guo *et al.* [29] found no significant increase in sensitivity with ROSE, but its use allowed performing fewer needle punctures and briefer procedures.

Two randomized-controlled trials exist in the literature that examined the role of ROSE during EBUS-TBNA. The earlier study found unequivocal evidence that ROSE was associated with a significantly lower need for additional bronchoscopic procedures and punctures [21].

Rapid on-site evaluation and lung cancer genotyping

The second randomized-controlled trial was carried out by Trisolini *et al.* [6] who designed their study to assess the influence of ROSE on the yield of EBUS-TBNA for a multigene molecular analysis of lung cancer samples. One hundred and twenty six patients with suspected or known advanced lung cancer were randomized to undergo EBUS-TBNA without ROSE or with ROSE. In addition to shortening the procedural time, ROSE prevented the need for a repeat invasive diagnostic procedure aimed at molecular profiling in at least one out of 10 patients and significantly reduced the risk of retrieving samples that can be used only for pathologic subtyping [6]. An important point to note in the former study was that only tissue cores retrieved during TBNA could be used for molecular testing, whereas cytology specimens were used for pathological diagnosis.

In a subsequent pivotal study by Casadio *et al.* [34], 306 patients with clinically diagnosed primary lung cancer underwent the EBUS-TBNA procedure, and the *EGFR* and *KRAS* mutations were evaluated this time on the cytological specimens produced. Although this study was not specifically evaluating the on-site cytology procedure, ROSE was central to their methodology. Molecular testing was only performed on the cytology if deemed adequate by ROSE. The authors concluded that EBUS-TBNA (when combined with ROSE) can be effectively used not only for diagnosis but also for complete mutational testing [34].

Rapid on-site evaluation in benign diseases

ROSE during EBUS-TBNA for patients with suspected sarcoidosis was prospectively studied by Plit *et al.* [16] who compared the diagnostic accuracy of EBUS-TBNA with ROSE with the final cytological assessment and with transbronchial and endobronchial biopsies in 60 patients. ROSE had high diagnostic accuracy (88%), and agreement with other modalities was present in 91% of cases. They concluded that ROSE can inform the bronchoscopist in theater whether additional diagnostic procedures need to be undertaken [16]. More recently, Rokadia *et al.* [31] retrospectively examined 255 cases with granulomatous disease as their final diagnosis who had undergone EBUS-TBNA with ROSE during their diagnostic workup. There was 81% concordance between the ROSE findings and the final diagnosis. The concordance was not impacted by needle size, lymph node size or station, number of stations biopsied, or passes per lymph node [31].

Recent innovations

Among the recent advances with ROSE was the introduction of the Bioevaluator (Murazumi Industrial Co. Ltd.; Osaka, Japan) system in a study by Minami *et al.* [25]. It is a device used for determining whether the tissues obtained by EBUS-TBNA are appropriate for a pathological diagnosis. A special light was used to examine the aspirated material after being smeared on a slide. Tissue areas appearing white and red through Bioevaluator were considered to be appropriate and inappropriate, respectively. Checking aspirated samples using this new system appeared useful for determining their adequacy for pathological diagnosis [25]. Another aspect that was explored was the use of telemedicine in ROSE. Real-time images of stained cytology smears were obtained using a digital camera attached to an Olympus microscope (Olympus; Tokyo, Japan) and transmitted through ethernet by a cytotechnologist to a cytopathologist in a cytopathology laboratory who rendered a preliminary diagnosis while communicating with an on-site cytotechnologist [23]. The overall concordance between the preliminary and final diagnoses was 96% for telecytopathology and 93% for conventional microscopy. It was concluded that telecytopathology is comparable with conventional microscopy in ROSE with EBUS-TBNA. It can serve as a valid substitute for conventional microscopy for on-site assessment of EBUS-TBNA [23].

Rapid on-site evaluation by the pulmonologist

A recent study tried to verify whether a pulmonologist with training in cytology can perform ROSE [24]. A total of 364 aspirations made by cTBNA were first examined through ROSE by a cytology-trained pulmonologist. These smears were later examined by a board-certified cytologist. There was an 81% overall substantial agreement between observers. The study was only designed to evaluate the feasibility of the concept, and thus the authors did not comment on the impact of ROSE on sensitivity or complications. The implications of this study are significant. Training pulmonologists to have a basic knowledge of cytopathology can possibly obviate most difficulties related to the involvement of cytopathologists in routine diagnostic activities and may reduce the costs of the procedure [24]. Performance of ROSE by the pulmonologist during both cTBNA and EBUS-TBNA has gained some popularity and is now performed routinely in many centers, especially in Europe.

Conclusion

Despite the overzealous outlook for the role of ROSE in TBNA in earlier studies, the accumulating evidence has confirmed its value for decreasing the number and variety of bronchoscopy sampling methods during both cTBNA and EBUS-TBNA. ROSE has shown acceptable sensitivity both for malignant and benign disease. The role of ROSE is emerging in molecular testing for lung cancer, and the capacity of pulmonologists to perform ROSE using telemedicine technology will serve to propagate the application of the procedure.

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Conflicts of interest

There are no conflicts of interest.

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