# Rapid cytological analysis of endobronchial ultrasound-guided aspirates in sarcoidosis

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ABSTRACT Rapid on-site evaluation (ROSE) of endobronchial ultrasound-guided transbronchial needle aspirates (EBUS-TBNA) has not been compared to final detailed cytological analysis in patients with suspected sarcoidosis.

To assess the diagnostic accuracy of EBUS-TBNA with ROSE in patients with suspected sarcoidosis, a prospective two-centre study performed EBUS-TBNA with ROSE of cellular material followed by transbronchial lung biopsy (TBLB) and endobronchial biopsy (EBB). The diagnostic accuracy of EBUS-TBNA with ROSE was compared to the final cytological assessment and to TBLB and EBB.

Analysis confirmed 49 out of 60 cases of sarcoidosis. ROSE sensitivity was 87.8% (specificity 91%, positive predictive value 97.7%). ROSE slide interpretation in combination with the final fixed slide and cell block preparations had a sensitivity of 91.8% (specificity 100%, positive predictive value 100%). 67% of patients were confirmed as having sarcoidosis on TBLB and 29% on EBB. Interobserver agreement between cytotechnologists and pathologists was very good ($\kappa$=0.91, 95% CI 0.80–1.0 and $\kappa$=0.91, 95% CI 0.79–1.0, respectively).

EBUS-TBNA with ROSE has high diagnostic accuracy and interobserver agreement and informs the bronchoscopist in theatre whether additional diagnostic procedures need to be undertaken. EBUS-TBNA with ROSE should therefore be considered as the first-line investigation of sarcoidosis.

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## Introduction

The 1999 American Thoracic Society/European Respiratory Society statement on sarcoidosis required that the diagnosis of sarcoidosis be confirmed by a compatible clinical picture, the histological identification of noncaseating granulomas and the exclusion of other diseases capable of producing a similar histological or clinical picture [1]. The value of a diagnostic test not only depends upon accuracy and predictability, but whether the test has the ability to change patient outcome. Clinical and radiological features alone may be diagnostic for sarcoidosis in up to 98% of cases and the disease runs a benign course in most patients [1, 2]. The ideal diagnostic procedure for patients with sarcoidosis should therefore have a high sensitivity, exclude other more serious diseases and have a very low complication rate that justifies an interventional procedure in patients with a high pre-test probability of having the disease, particularly if it can be undertaken rapidly as a day procedure. The traditional approach to confirming the diagnosis of sarcoidosis has presumed histology to be the gold standard and has not ideally fulfilled these conditions. In a large meta-analysis, mediastinoscopy was associated with a median complication rate of 2% and was highly dependent on the experience and skill of the surgeon [3]. The diagnostic yield of transbronchial lung biopsies (TBLB), often taken with endobronchial biopsies (EBB), is highly variable and operator dependent, ranging from 32% to 100% [4–10], and is associated with a pneumothorax rate of $\geqslant$1.4% and haemorrhage in 4% of patients, even in units that undertake this procedure in large numbers of patients [11]. Bronchoalveolar lavage is a low-risk investigational tool, but has a sensitivity of only 53–59% for sarcoidosis [12]. Endobronchial ultrasound-guided transbronchial needle aspirate (EBUS-TBNA) potentially fulfils all the requirements of an ideal diagnostic test in patients with suspected sarcoidosis. Single-centre studies have reported diagnostic yields of 83–94% and complication rates of $<$1% [10, 13–20]. Recent studies concluded that EBUS-TBNA in combination with TBLB and other standard bronchoscopic techniques optimises the diagnostic yield and should be considered the first-line investigation in patients with suspected sarcoidosis [10, 17, 19]. Alternatively, the decision to proceed to TBLB after EBUS-TBNA was left up to the discretion of the bronchoscopist [13]. Rapid on-site evaluation (ROSE) of cytological material assists the EBUS-TBNA procedure by informing the bronchoscopist about the number of lymph node stations and passes that are required, and whether TBLB and EBB need to be undertaken at the same session rather than bringing the patient back when the final cytological or histological assessment has been completed [10, 13, 14, 18]. This study is the first prospective, blinded study to assess the diagnostic accuracy and interobserver agreement between cytotechnologists of EBUS-TBNA with ROSE in patients with suspected sarcoidosis.

## Methods

The study was planned according to the ethics guidelines of the Helsinki Declaration, and study protocol approved by St Vincent's Hospital (Sydney, Australia) and the Royal Brisbane Hospital (Brisbane, Australia) human research and ethics committees. In order to address recommendations in the literature, the inclusion criteria were identical to recent studies and we consecutively recruited patients with suspected sarcoidosis on the basis of a typical clinical presentation and computed tomography (CT) evidence of hilar and/or mediastinal lymphadenopathy with or without lung infiltrates. Patients were excluded from the study if there were systemic symptoms, including weight loss, fever or radiological lesions suggestive of lung cancer or tuberculosis. Each patient underwent EBUS-TBNA, TBLB and EBB as a day surgery procedure, as previously described by our group [16]. Each procedure was undertaken under general anaesthesia with a laryngeal mask airway and intravenous fentanyl and propofol. All EBUS-TBNA samples were obtained using 22-gauge needles. Each bronchoscopist was highly experienced and was accredited in a unit which routinely performs more than 600 bronchoscopic procedures annually.

The lymph node stations were identified according to the International Staging Classification [21]. More than half the smears were air-dried and a rapid Romanowsky-type stain (Diff-Quik Stain; Australian Biostain Pty Ltd, Traralgon, Australia) was applied, while the other smears were immediately fixed in 95% ethyl alcohol for later staining using the Papanicolaou (Pap) technique [22]. The air-dried Diff-Quik-stained smears were rapidly evaluated by the on-site cytotechnologist. The needles and syringes were rinsed in saline and immediately placed in RPMI for cell block preparation. Cellular material was sent for flow cytometry if lymphoma was suspected. If tuberculosis was suspected aspirated material was also sent for acid-fast stains, mycobacterial culture and PCR. Mycobacterial cultures were incubated for 8 weeks. If malignant cells were recognised, cellular material was referred for tumour marker analysis and neither TBLB nor EBB was undertaken.

The decision to proceed to additional lymph node passes was therefore guided by the cytotechnologist's assessment of the adequacy of the aspirated cellular material as defined by the presence of granulomas, germinal centre fragments, malignant cells or abundant lymphocytes in multiple low-power fields. Inadequate sampling of a lymph node was defined as the absence of lymphoid material or presence of excessive bronchial cell contamination, necrotic tissue or blood [22, 23]. The final diagnosis of sarcoidosis

was based on the pathological interpretation of all received material by the presence of well-formed noncaseating epithelioid granulomas and the absence of microscopic evidence of mycobacteria on special staining [1]. Anthracosis was diagnosed when there were aggregated sheets and relatively poorly formed granulomas consisting of carbon pigment-laden macrophages in the presence of compatible clinical and radiological features.

TBLB was performed under fluoroscopic control, and a total of eight to 10 biopsies of $\geqslant$1–2 mm diameter from the middle and lower lobes were obtained to ensure adequate sampling. Four endobronchial biopsies of airway mucosa were then undertaken from abnormal appearing bronchial mucosa or at any subcarinal location if there was no mucosal abnormality. A chest radiograph was performed 3 h post-procedure to exclude a pneumothorax and the patient was discharged after 4–6 h if no complications had occurred.

In each hospital, the pathologist interpreting the EBUS-TBNA material was blinded to the results of the histopathological interpretation of the TBLB and the EBB, as well as the initial cytotechnologist's interpretation of the ROSE slides. After finalisation of the cytopathological reports, the slides were then couriered to the other participating hospital for a blinded re-evaluation of the material.

### *Statistical analysis*

The data were analysed using SPSS (SPSS Inc., Chicago, IL, USA). As the published diagnostic yield of TBLB has ranged from 32% to 100% [4–10], we calculated that $\geqslant$58 patients were required to have an 80% chance (1-β) of detecting a significant ($p<0.05$) 30% difference in primary outcome measure between TBLB and EBUS-TBNA. The diagnostic accuracy rate was calculated according to standard definitions. A two-tailed Fisher's exact test for categorical variables using a $2\times2$ contingency table was employed to compare diagnostic accuracy rates between the groups. Significant difference between groups was set at $p\leqslant0.05$. Interobserver agreement between the cytotechnologists at the two hospitals and also between the pathologists at the two hospitals was quantified by calculating a κ-score.

## Results

60 consecutive patients were prospectively recruited into the study at the two participating hospitals between July 2010 and July 2011. The characteristics of the patients are listed in table 1. The number of node aspirates was guided by the on-site assessment by the cytotechnologist of the rapidly air-dried slides. When a pass showed clearly recognisable granulomas, at least two additional passes were undertaken for cell block and mycobacterial cultures. If the initial pass was nondiagnostic but revealed abundant lymphocytes compatible with adequate lymph node sampling, at least one other node was sampled before proceeding to TBLB and EBB. A total of 90 lymph nodes were aspirated, with an average of four passes per node (mean (range) 1.5 (2–11) lymph nodes per patient, including 52 subcarinal, 15 paratracheal, 17 right hilar and six left hilar nodes). The lymph nodes had a median (range) diameter of 16 (8–42) mm. A typical well-formed granuloma (as per the on-site preparation) is demonstrated in figure 1.

Of the 60 patients, a final diagnosis of sarcoidosis was made in 49 patients utilising a combination of EBUS-TBNA, TBLB and EBB. 11 patients had a variety of other diagnoses which are listed in table 1 and figure 2. Granulomas were clearly recognised on the initial EBUS-TBNA ROSE slides as reported by the on-site cytotechnologist in 46 patients. One of these patients was subsequently shown to have caseating granulomas and acid-fast bacilli only on the Pap and auramine slides. *Mycobacterium intracellulare* was isolated on

TABLE 1 Characteristics of the combined study population from the participating hospitals

| | |
|---|---|
| **Subjects** | 60 |
| **Age years** | 47±12.2 |
| **Males %** | 57 |
| **Stage I sarcoidosis**[#] | 23 |
| **Stage II sarcoidosis**[¶] | 26 |
| **Other diagnosis** | 11 |
| Nonsmall cell lung cancer | 1 |
| Hodgkin's disease | 1 |
| Anthracosis | 3 (2 anthracotic granulomas on ROSE TBNA, 1 on TBLB only) |
| *Mycobacterium intracellulare* | 1 |
| *Haemophilus influenzae* pneumonia | 1 |
| Reactive | 4 (1 node with psammoma bodies) |

Data are presented as n or mean±SD, unless otherwise stated. ROSE: rapid on-site evaluation; TBNA: transbronchial needle aspirates; TBLB: transbronchial lung biopsies. [#]: hilar adenopathy only; [¶]: hilar lymphadenopathy plus lung infiltrates.


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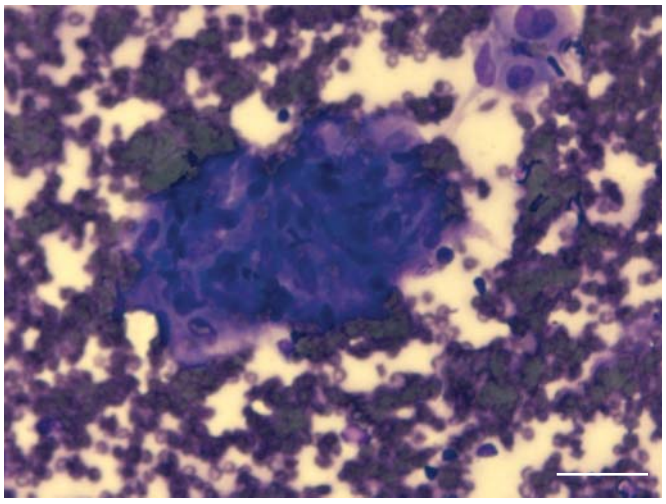

FIGURE 1 A granuloma, consisting of epithelioid histiocytes, identified in theatre using the Diff-Quik stain (Australian Biostain Pty Ltd, Traralgon, Australia) (rapid Romanowsky-type stain). Scale bar=30 μm.

culture of a lymph node aspirate. This finding was retrospectively regarded as a false-positive result for sarcoidosis by ROSE. Two other patients had poorly formed granulomas with prominent carbon deposition and were thought to be compatible with a diagnosis of anthracosis. As the anthracotic granulomas were readily recognised on the ROSE slides, they were regarded as true negative results.

The sensitivity of the ROSE slide interpretation for sarcoidosis was therefore 87.8% (43 out of 49; 95% CI 0.76–0.95), specificity was 90.9% (10 out of 11) and positive predictive value was 97.7% (43 out of 44). When the ROSE slide interpretation was combined with the final fixed slides (Pap and acid-fast stains), cell block preparations and microbiological testing after the patient was discharged from the theatre, the sensitivity of EBUS-TBNA was 91.8% (45 out of 49; 95% CI 0.83–0.99), specificity was 100% (nine out of

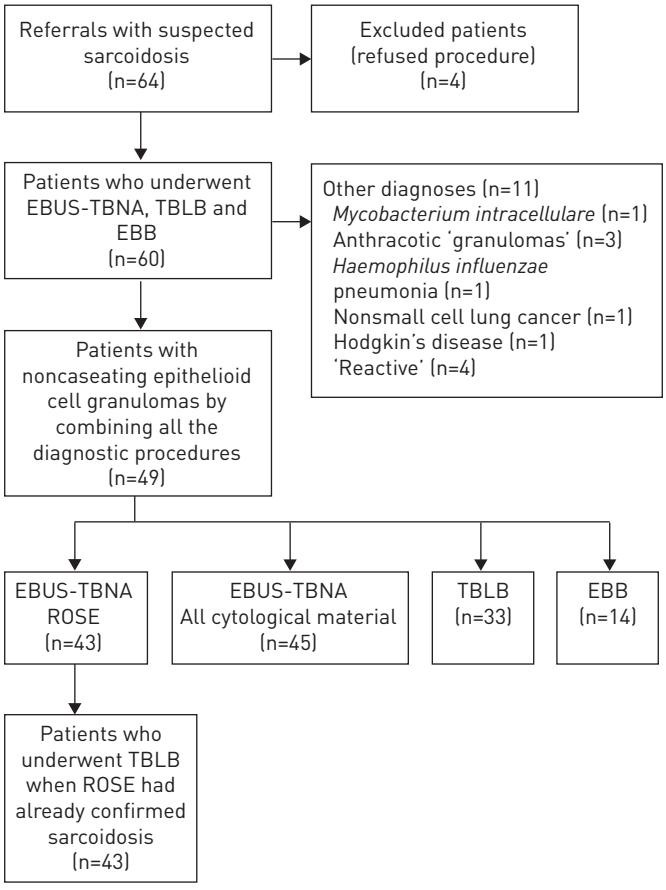


FIGURE 2 Flow diagram illustrating the diagnostic pathway of patients with suspected sarcoidosis referred for entry into the study. EBUS-TBNA: endobronchial ultrasound-guided transbronchial needle aspirate; TBLB: transbronchial lung biopsy; EBB: endobronchial biopsy; ROSE: rapid on-site evaluation.

TABLE 2 Comparisons of diagnostic procedures for sarcoidosis

| | Positive results | Sensitivity | Specificity | Positive predictive value | Negative predictive value |
|---|---|---|---|---|---|
| **EBUS-TBNA with ROSE** | 43/49 | 87.8 (43/49) | 90.9 (10/11) | 97.7 (43/44) | 62.5 (10/16) |
| **EBUS-TBNA (final slides)#** | 45/49 | 91.8 (45/49) | 100.0 (9/9) | 100.0 (45/45) | 73.3 (11/15) |
| **TBLB** | 33/49 | 67.3 | 100.0 (11/11) | 100.0 (33/33) | 40.7 (11/27) |
| **EBB** | 14/49 | 28.6 | 100.0 (11/11) | 100.0 (14/14) | 23.9 (11/46) |

Data are presented as n/N, % or % (n/N). EBUS-TBNA: endobronchial ultrasound-guided transbronchial needle aspirate; ROSE: rapid on-site evaluation; TBLB: transbronchial lung biopsy; EBB: endobronchial biopsy. #: final slides include Diff-Quik, Papanicolaou and auramine stains and cell block preparations examined in the laboratory by a cytopathologist. EBUS-TBNA *versus* TBLB $p=0.29$; EBUS-TBNA *versus* EBB $p<0.01$.

nine) and positive predictive value was 100% (45 out of 45) (table 2). The strength of diagnostic agreement between the cytotechnologists at the two participating hospitals when they reviewed each other's ROSE slides according to the blinded protocol was very good, generating a κ-score of 0.91 (95% CI 0.8–1.0). The strength of diagnostic agreement between the pathologists at the two respective hospitals after reciprocal re-examination of all the cellular material including the ROSE, fixed stains and the cell blocks was also very good ($\kappa=0.91$, 95% CI 0.79–1.0). The level of agreement between the cytotechnologists' interpretation of the ROSE slides and the pathologists' final evaluation was good ($\kappa=0.86$, 95% CI 0.65–0.99), even though the latter utilised all available material including the Diff-Quik stains, the Pap stains and the cell block.

Only 33 (67.3%) out of 49 (95% CI 0.53–0.79) patients were confirmed as having sarcoidosis on TBLB (table 2). There was no significant difference in diagnostic accuracy of TBLB between stage I and stage II sarcoidosis ($p=0.86$). EBUS-TBNA had a higher diagnostic accuracy for sarcoidosis than TBLB but this was not significantly different ($p=0.29$). The diagnostic accuracy of EBB for sarcoidosis was 14 (28.6%) out of 49 (95% CI 0.18–0.43), which was significantly worse than TBLB ($p=0.03$). TBLB and EBB each added to the diagnosis obtained on EBUS-TBNA alone by 4%. TBLB was complicated by bleeding of 50–100 mL in 5% of patients and a pneumothorax rate of 8%. Four out of the five patients had minimal, subclinical apical pneumothoraxes which did not require chest tube drainage. EBUS-TBNA did not result in any complications. The mean±SD duration of the EBUS-TBNA in combination with TBLB and EBB was 49.2±11.5 min in this study. This compares with an average duration of EBUS-TBNA alone of 20 min in our units. Each patient has been followed up for ⩾12 months, during which time no clinical features that would suggest that the pathological diagnosis of sarcoidosis was incorrect have developed.

## Discussion

This dual-centre prospective study provides compelling evidence that EBUS-TBNA with ROSE correlates well with the final overall pathological assessment and has high interobserver agreement between cytotechnologists. It therefore challenges recent literature that recommends that EBUS-TBNA should be combined with TBLB in patients with suspected sarcoidosis [10, 13, 17, 19]. Some studies have used cytopathologists for EBUS-TBNA with ROSE [10, 13, 14] in patients with suspected sarcoidosis, but did not utilise the diagnostic information to enable a decision to be made not to proceed to TBLB. This study confirms a growing body of evidence in recent years that shifts the paradigm from the primacy of histology in the diagnosis of sarcoidosis to the importance of cytological assessment [24]. Fine-needle cytology is still underutilised in the diagnosis of sarcoidosis [25]. TRISOLINI *et al.* [26] showed that fine-needle cytology often yields more material than that of histological samples in both patient-based (79% *versus* 30%) and procedure-based (70% *versus* 22.5%) analyses. EBUS-TBNA utilising 21- or 22-gauge needles does not usually provide ''core'' samples. However, aspirated cellular material can be utilised to create a cell block which represents a ''melange'' of cells and has been reported to add to the diagnostic yield of granulomas obtained from conventional cytological evaluation [27]. We utilised the Diff-Quick stain for ROSE rather than a rapid Pap stain. Unfortunately, Pap takes too long in the EBUS setting. Furthermore, recognition of granulomas is more difficult on a Pap stain compared to the haematological stain. However, each institution can use their preferred stain, but ROSE has to be quick and the Diff-Quik or similar is much faster than a rapid Pap.

The initial diagnostic yield of EBUS-TBNA with ROSE was 87.8% and after further analysis of the Diff-Quik stains, as well as the additional stains in the laboratory, the diagnostic yield increased to 91.8%. The latter diagnostic yield is comparable to other studies utilising ROSE [10, 13, 14]. Studies that have not utilised


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ROSE have reported a diagnostic yield of 83–85% [16, 19] except for one recent study which achieved a high diagnostic yield of 94% [20]. However, the purpose of our study was not to demonstrate that EBUS-TBNA with ROSE has a higher diagnostic yield compared to standard cytological processing, but rather to answer the question whether EBUS-TBNA with ROSE provides a sufficiently robust diagnostic yield to inform the bronchoscopist whether additional lymph node passes or TBLB need to be undertaken prior to the patient leaving theatre. In our study 43 (72%) out of 60 patients underwent TBLB even after ROSE had already confirmed sarcoidosis. 46 (77%) out of 60 patients therefore underwent unnecessary TBLB when the ROSE also confirmed cancer or anthracosis (fig. 2). Furthermore, the unexpected identification of metastatic nonsmall cell lung cancer on ROSE in one patient enabled the collection of additional cellular material for immunohistochemistry and molecular markers. In contrast, only four (8%) patients with nondiagnostic ROSE had sarcoidosis confirmed on TBLB or EBB, thus justifying the undertaking of additional procedures in this subgroup of patients. EBUS-TBNA with ROSE not only prevented the need to undertake unnecessary TBLB, but informed the bronchoscopist at the same session when additional passes and procedures were likely to benefit the patient. These patients were spared the inconvenience, risk and cost of a subsequent return to theatre.

Our study highlights the valuable role of well-trained cytotechnologists in patients with suspected sarcoidosis, which is particularly important if centres are unable to obtain the services of a cytopathologist in theatre. The cytotechnologists in this study are university biomedical science graduates who have completed a minimum of four postgraduate years in cytology and passed the Certificate of Cytotechnology of the Australian Society of Cytology and the International Academy of Cytology Cytotechnician's Certificate. However, our cytotechnologists had no special expertise in sarcoidosis and the high intra- and interobserver agreement between the cytotechnologists and between the cytotechnologists and cytopathologists from different hospitals suggests that their high diagnostic accuracy with ROSE is generally applicable to other bronchoscopy units. The cost saving in using the services of a cytotechnologist rather than a pathologist in theatre is considerable. In Australia a cytotechnologist is paid AUD 35 per hour, as opposed to a pathologist receiving AUD 180 per hour. In addition, the attending fee of a pathologist is AUD 180 for a single site. Institutions that have not utilised ROSE attempt to assess the quality of the sample by gross visual inspection. However, there is no evidence to validate this approach with EBUS-TBNA, although it is a relatively poor technique in patients with endoscopic ultrasound-guided aspiration of pancreatic masses [28].

Our TBLB diagnostic rate for sarcoidosis for all stages was 67% (33 out of 49) and as high as 78% (18 out of 23) for stage I where there is a clinical expectation of a low yield when there is no CT scan evidence of lung infiltrates. Reports of diagnostic yield from TBLB for stage I disease are highly variable and operator dependent, ranging from 32% to 100% [5, 7, 9, 10, 18]. ROETHE *et al.* [9] were the first to suggest that a high diagnostic yield can be obtained from TBLB even in stage I. They reported a 100% diagnostic yield from 10 biopsies in stage I disease. The diagnostic accuracy of TBLB therefore depends on the number of biopsies that are taken. The diagnostic yield from TBLB for stage II sarcoidosis in our study was only 58% (15 out of 26), which is relatively low, with a reported diagnostic yield in the literature of 63–100% [5–7, 10, 18]. This may be a chance finding or may represent the fact that this study was undertaken in two hospitals where TBLB technique may be different despite using the same study protocol. Although sarcoidosis has typical upper lobe predominance radiologically, our previous study [18] revealed a high diagnostic yield of 80% when biopsies were taken from middle and lower lobes. The pneumothorax rate following TBLB in our study was higher than in other studies and, in fact, the incidence was higher than our previously reported experience in lung transplant and sarcoidosis patients [11, 18]. The most plausible explanation for this discrepancy is that we undertook between eight and 10 biopsies in each patient in order to maximise diagnostic yield and each sample was only considered adequate if the diameter was $\geqslant$1–2 mm. Most other recent studies took only four to six biopsies [10, 19, 20, 29] or failed to report the number of biopsies in each patient [17]. Four out of five of the patients had minimal, subclinical apical pneumothoraces which did not require chest tube drainage. Every patient had a chest radiograph post-procedure, even if they were asymptomatic, and a meticulous examination of the apices was undertaken. However, our study was unfortunately not designed to determine whether this discrepancy was a chance finding. EBB had a low diagnostic accuracy of 29%, which is very similar to our previous study which reported a diagnostic yield of 27% [18]. SHORR *et al.* [8] reported EBB alone to have a diagnostic yield of 61.8%. In their study EBB findings were more frequently positive in abnormal appearing airways. However, biopsy of normal appearing bronchial mucosa provided diagnostic tissue in 30% of their patients [8]. This is similar to our cohort, in which the majority of patients had normal appearing mucosa. EBUS-TBNA did not result in any complications.

Our study clearly demonstrates that EBUS-TBNA with ROSE provides a high and reproducible diagnostic yield and this immediately informs the bronchoscopist in theatre as to whether additional lymph node

passes or TBLB need to be undertaken. EBUS-TBNA with ROSE therefore provides sufficiently robust diagnostic information and a safety profile that consolidates its role as the first-line investigation in patients with suspected sarcoidosis.

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