Rapid Cytological Analysis of Endobronchial Ultrasound-Guided Aspirates in Sarcoidosis

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Detail of authorship contributions: all listed authors have contributed to study conception and design, data acquisition or analysis and interpretation of data. All authors have either drafted or revised the article and have reviewed the article for final approval prior to publication.

Funding Source: Funding for this study has been sourced from a research trust supported by private donations. No funding has been obtained from any commercial or pharmaceutical enterprise.

Word Count: 3133

Short Running Head: Rapid Evaluation of EBUS-Guided Aspirates
Abstract

Rationale: Rapid on site evaluation (ROSE) of Endobronchial ultrasound-guided transbronchial needle aspiration (EBUS-TBNA) has not been compared to the final detailed cytological analysis in patients with suspected sarcoidosis.

Objective: To assess the diagnostic accuracy of EBUS-TBNA with ROSE in patients with suspected sarcoidosis.

Methods: A prospective two-centre study undertook EBUS-TBNA with ROSE of cellular material by cytotechnologists followed by TBLB and EBB. The diagnostic accuracy of EBUS-TBNA with ROSE was compared to the final cytological assessment as well as TBLB and EBB.

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

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

Abstract Word Count: 199

Key Words: sarcoidosis, endobronchial ultrasound, diagnostic tool
Introduction

The American Thoracic Society Statement in 1999 required that the diagnosis of sarcoidosis be confirmed by a compatible clinical picture, the histological identification of non-caseating granulomas and exclusion of other diseases capable of producing a similar histologic 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. The 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 as 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, 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 at least 1.4% and haemorrhage in 4% of patients even in units which undertake this procedure in large numbers of patients (11). Bronchoalveolar lavage (BAL) is a low-risk investigational tool but has a sensitivity of only 53 to 59% for sarcoidosis (12). Endobronchial ultrasound-guided transbronchial needle aspirates (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% to 94% and complication rates of < 1% (10, 13-20). Recent studies concluded that EBUS-TBNA in combination with TBLB and other standard bronchoscopic techniques optimizes 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 regarding the number of lymph node stations and passes that are required and whether TBLB and EB 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 report is the first prospective, blinded study which assessed 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 the St Vincent’s Hospital, Sydney and Royal Brisbane Hospital 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 CT scan 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 utilizing 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) was applied, while the other smears were immediately fixed in 95% ethyl alcohol for later staining by the Papanicolaou technique (Pap). (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 recognized, 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 non-caseating 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 8-10 biopsies of at least 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 X-ray was performed three hours post procedure to exclude a pneumothorax and the patient was discharged after 4-6 hours 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 finalization 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). As the published diagnostic yield of TBLB has ranged from 32 to 100% (4-10), we calculated that at least 58 patients were required to have an 80% chance (1-beta) 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 2x2 contingency table was employed to compare diagnostic accuracy rates between the groups. Significant difference between groups was set at p ≤ 0.05. Interobserver
agreement between the cytotechnologists at the two hospitals and also between the pathologists at the two hospitals was quantified by calculating a kappa score.

**Results**

60 consecutive patients were prospectively recruited into the study at the two participating hospitals between July 2010 and July 2011. The patient characteristics are listed in Table 1. The number of node aspirates was guided by the on-site assessment by the cytotechnologist of the rapid air-dried slides. When a pass showed clearly recognizable granulomas, at least 2 additional passes were undertaken for cell block and mycobacterial cultures respectively. If the initial pass was non-diagnostic 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 4 passes per node (mean of 1.5 lymph nodes per patient, range of (2-11) including 52 subcarinal, 15 paratracheal, 17 right hilar and 6 left hilar nodes). The lymph nodes had a median diameter of 16mm (range 8mm – 42mm). 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 utilizing a combination of EBUS-TBNA, TBLB and EBB. Eleven patients had a variety of other diagnoses and are listed in Table 1. Granulomas were clearly recognized 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 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 recognized on the ROSE slides, they were regarded as ‘true negative’ results.

The sensitivity of the ROSE slide interpretation for sarcoidosis was therefore 87.8% (43/49), (95% CI 0.76 to 0.95), specificity of 91% (10/11) and positive predictive value 97.7% (43/44). 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% (45/49), (95% CI 0.83-0.99), specificity 100% (9/9), and positive predictive value was 100% (45/45) (Table 2). The strength of diagnostic agreement between the cytotechnologists at the two participating hospitals when they reviewed each others’ ROSE slides according to the blinded protocol was very good generating a Kappa 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 cytotechnologist’s interpretation of the ROSE slides and the pathologist’s final evaluation was good (Kappa = 0.86, 95% CI = 0.65-0.99) even though the latter utilized all available material including the Diff-Quik stains, the Pap stains and the cell block.

Only 33/49 (67%), (95% CI 0.53 - 0.79) of patients were confirmed as having sarcoidosis on TBLB (Table 2). There was no significant difference in diagnostic accuracy of TBLB between stage 1 and stage 2 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 14/49 (29%), (95% CI 0.18 to 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 between 50-100mls in 5% of patients and a pneumothorax rate of 8%. 4/5 of the patients had minimal, subclinical apical pneumothoraxes which did not require chest tube drainage. EBUS-TBNA did not result in any complications. The mean duration of the EBUS-TBNA in combination with TBLB and EBB was 49.2 minutes (+/- 11.5) in this study. This compares with an average duration of EBUS-TBNA alone of 20 minutes in our units. Each patient has been followed up for at least 12 months during which time no clinical features have developed which would suggest that the pathological diagnosis of sarcoidosis was incorrect.

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 and 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 utilized cytopathologists for EBUS-TBNA with ROSE (10, 13, 14) in patients with suspected sarcoidosis but did not utilize 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 (FNC) is still underutilized in the diagnosis of sarcoidosis (25). Trisolini showed that FNC often yields more material than that of histological samples in both the patient-
based (79% versus 30%) and the procedure-based analysis (70% vs 22.5%) (26).
EBUS-TBNA utilizing 21 or 22 gauge needles does not usually provide "core" samples. Aspirated cellular material can however be utilized 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 utilized the Diff-Quick stain for ROSE rather than a rapid Papanicolaou stain. Pap unfortunately takes too long in the EBUS setting. Furthermore, recognition of granulomas is more difficult on a Pap stain compared to the 'hematological' stain. However, each institution can use whatever stain they prefer, 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 utilizing ROSE (10, 13, 14). Studies that have not utilized ROSE have reported a diagnostic yield of 83-85% (16, 19) except for one recent study that achieved a high diagnostic yield of 94% (20). The purpose of our study was however not intended to demonstrate that EBUS-TBNA with ROSE has a higher diagnostic yield as compared to standard cytological processing but rather to answer the question as to whether EBUS-TBNA with ROSE provides a sufficiently robust diagnostic yield to inform the bronchoscopist as to whether additional lymph node passes or TBLB need to be undertaken prior to the patient leaving theatre. In our study 43/60 patients (72%) underwent TBLB even after ROSE had already confirmed sarcoidosis. 46/60 patients (77%) therefore underwent unnecessary TBLB when the ROSE also confirmed cancer or anthracosis (Figure 2). Furthermore, the unexpected identification of metastatic non-small cell lung cancer (NSCLC) on ROSE in 1 patient
enabled the collection of additional cellular material for immunohistochemistry and molecular markers. In contrast only 4 (8%) of patients with non-diagnostic ROSE had sarcoidosis confirmed on TBLB or EBB thus justifying the need to undertake 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 Australian Society of Cytology Cytotechnician’s Certificate and the International Society of Cytology Cytotechnician’s Certificate. However our cytotechnologists had no special expertise in sarcoidosis and the high 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 utilizing the services of a cytotechnologist rather than a pathologist in theatre is considerable. In Australia a cytotechnologist is paid $35 dollars an hour as opposed to a pathologist receiving $180 per hour. In addition, the attending fee of a pathologist is $180 for a single site. Institutions that have not utilized ROSE attempt to assess the quality of the sample by gross visual inspection. There is however 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% (33/49) and as high as 78% for stage I (18/23) 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 1 disease are highly variable and operator dependent ranging from 32% to 100% (5, 7, 9, 10, 18). Roethe et al was the first group to suggest that a high diagnostic yield can be obtained from TBLB even in stage 1. They reported a 100% diagnostic yield from 10 biopsies in stage 1 disease (9). 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% (15/26) which is relatively low with a reported diagnostic yield in the literature of 63-100% (5, 6, 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 in spite of using the same study protocol. Although sarcoidosis has typical upper lobe predominance radiologically, our previous study 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 8-10 biopsies in each patient in order to maximize diagnostic yield and each sample was only considered adequate if the diameter was at least 1-2mm in diameter. Most other recent studies took only 4-6 biopsies (10, 19, 20, 29) or failed to report the number of biopsies in each patient (17). 4/5 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 or not. 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 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.
<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Count</th>
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<tbody>
<tr>
<td>Number</td>
<td>60</td>
</tr>
<tr>
<td>Age (yr), mean (SD)</td>
<td>47(12.2)</td>
</tr>
<tr>
<td>Gender, % male</td>
<td>57</td>
</tr>
<tr>
<td>Stage 1 sarcoidosis *</td>
<td>23</td>
</tr>
<tr>
<td>Stage 2 sarcoidosis †</td>
<td>26</td>
</tr>
<tr>
<td>Other diagnosis</td>
<td>11</td>
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<tr>
<td>Non-small cell lung cancer X 1</td>
<td></td>
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<tr>
<td>Hodgkin’s Disease X 1</td>
<td></td>
</tr>
<tr>
<td>Anthracosis X 3 (anthracotic granulomas x 2 on ROSE TBNA, 1 on TBLB only)</td>
<td></td>
</tr>
<tr>
<td>Mycobacterium intracellulare X 1</td>
<td></td>
</tr>
<tr>
<td>Hemophilus influenzae pneumonia X1</td>
<td></td>
</tr>
<tr>
<td>Reactive X 4, (1 node with psammoma bodies)</td>
<td></td>
</tr>
</tbody>
</table>

Stage 1* = hilar adenopathy only; Stage 2† = hilar lymphadenopathy plus lung infiltrates.

ROSE – rapid on-site evaluation, TBNA – transbronchial needle aspirates; TBLB-transbronchial lung biopsies
Figure 1. A. Granuloma, consisting of epithelioid histiocytes, identified in theatre using the *Diff Quik Stain* (rapid Romanowsky-type stain). Original magnification: ×40.
<table>
<thead>
<tr>
<th>Procedure</th>
<th>Positive results</th>
<th>Sensitivity</th>
<th>Specificity</th>
<th>Positive predictive value</th>
<th>Negative predictive value</th>
</tr>
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<tbody>
<tr>
<td>EBUS-TBNA with ROSE*</td>
<td>43/49</td>
<td>87.8%</td>
<td>91%</td>
<td>97.7%</td>
<td>62.5%</td>
</tr>
<tr>
<td></td>
<td>(43/49)</td>
<td>(10/11)</td>
<td>(43/44)</td>
<td></td>
<td>(10/16)</td>
</tr>
<tr>
<td>EBUS-TBNA (final slides)#</td>
<td>45/49</td>
<td>91.8%</td>
<td>100%</td>
<td>100%</td>
<td>73.3%</td>
</tr>
<tr>
<td></td>
<td>(45/49)</td>
<td>(9/9)</td>
<td>(45/45)</td>
<td></td>
<td>(11/15)</td>
</tr>
<tr>
<td>TBLB‡</td>
<td>33/49</td>
<td>67%</td>
<td>100%(11/11)</td>
<td>100%(33/33)</td>
<td>41%(11/27)</td>
</tr>
<tr>
<td>EBB‡</td>
<td>14/49</td>
<td>29%</td>
<td>100%(11/11)</td>
<td>100%(14/14)</td>
<td>24%(11/46)</td>
</tr>
</tbody>
</table>

* EBUS-TBNA - Endobronchial ultrasound-guided transbronchial needle aspirate with Rapid-On-Site-Evaluation of cytological material

# Final slides include Diff-Quik, Papanicolaou and Auramine stains and Cell block preparations examined in the laboratory by a cytopathologist.

† TBLB – Transbronchial lung biopsy

‡ EBB - Endobronchial biopsy

EBUS - TBNA vs. TBLB, p = 0.29

EBUS TBNA vs. EB; p < 0.01
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 Onsite Evaluation.
References


