Endosonography-guided fine-needle aspiration in the diagnosis of sarcoidosis: a randomized study

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INTRODUCTION
There are no widely accepted standards for the diagnosis of sarcoidosis.

OBJECTIVES
The aim of this study was to assess the relative diagnostic yield of endobronchial ultrasound fine-needle aspiration (EBUS-FNA) and endoscopic ultrasound fine-needle aspiration (EUS-FNA), and to compare them with standard diagnostic techniques such as endobronchial biopsy (EBB), transbronchial lung biopsy (TBLB), transbronchial needle aspiration (TBNA), and mediastinoscopy.

PATIENTS AND METHODS
This was a prospective randomized study including consecutive patients with clinical diagnosis of stage I or II sarcoidosis. EBB, TBLB, and TBNA were performed at baseline in all patients. Subsequently, patients were randomized to group A (EBUS-FNA) or group B (EUS-FNA). Next, a crossover control test was performed: all patients with negative results in group A underwent EUS-FNA and all patients with negative results in group B underwent EBUS-FNA. If sarcoidosis was not confirmed, mediastinoscopy was performed.

RESULTS
We enrolled 106 patients, of whom 100 were available for the final analysis. The overall sensitivity and accuracy of standard endoscopic methods were 64% each. When analyzing each of the standard endoscopic methods separately, the diagnosis was confirmed with EBB in 12 patients (12%), with TBLB in 42 patients (42%), and with TBNA in 44 patients (44%). The sensitivity and accuracy of each endosonographic technique were significantly higher than those of EBB+TBLB+TBNA (P = 0.0112 vs P = 0.0134).

CONCLUSIONS
The sensitivity and accuracy of EBUS-FNA and EUS-FNA are significantly higher than those of standard endoscopic methods. Moreover, the sensitivity and accuracy of EUS-FNA tend to be higher than those of EBUS-FNA.
Endobronchial ultrasound needle aspiration of lymph node station 4R, showing the biopsy needle within the node (arrow)

Techniques have been used, including endobronchial biopsy (EBB), transbronchial lung biopsy (TBLB), transbronchial needle aspiration (TBNA), and mediastinoscopy. Recently, endobronchial ultrasound fine-needle aspiration (EBUS-FNA) and endoscopic ultrasound fine-needle aspiration (EUS-FNA) have been introduced.

**Patients and Methods**
The study was performed in the Department of Thoracic Surgery, Jagiellonian University Medical College, John Paul II Hospital, Kraków, Poland. The study design was approved by the Local Bioethics Committee (79/KBL/OIL/2009). All patients received detailed information regarding the study. The procedures, including the expected results and potential risks, were discussed with patients, and their informed consent was obtained.

It was a prospective randomized study including a consecutive group of patients with suspected stage I or II sarcoidosis, who were referred to the Department of Thoracic Surgery between October 2009 and May 2012.

The clinical questions of the study were: 1) what is the diagnostic yield of the standard bi- optic techniques (ie, EBB, TBLB, and TBNA) versus the endosonography-guided techniques (ie, EBUS-FNA and EUS-FNA) in diagnosing sarcoidosis, and 2) which of these 2 endosonography-guided techniques is more accurate in diagnosing sarcoidosis.

The inclusion criteria were as follows: age over 18 years, clinical diagnosis of stage I or stage II sarcoidosis, and signed written informed consent. The exclusion criteria were lack of patient’s consent to undergo endoscopic biotic techniques or mediastinoscopy, contraindications to bronchoscopy (eg, respiratory insufficiency or tracheal stenosis), coagulation disorders, and pregnancy.

Patients were randomized in a ratio of 1:1, using a computerized random digit generator in 2 groups. In group A, EBUS-FNA was performed, and in group B, EUS-FNA. In both groups, the standard diagnostic tests were performed during the same procedure, including EBB, TBLB, TBNA, and BACTEC Mycobacteria Growth Indicator Tube System analysis of the bronchial lavage. Patients were enrolled and randomized by 2 team members (PK or AS). All the above tests were performed during the same procedure.

In patients in whom these studies failed to confirm the diagnosis, a second endosonography-guided biotic technique was utilized in a crossover fashion (ie, EUS-FNA in those in whom EBUS-FNA was performed initially and EBUS-FNA in those in whom EUS-FNA was performed initially).

If the second endosonography-guided biotic technique failed to confirm the diagnosis, patients were scheduled for mediastinoscopy.

The recruitment of participants was stopped after inclusion of the planned number of 100 patients. All patients were followed for at least 6 months.

Endoscopic examination was performed using conscious sedation with midazolam and fentanyl, and local administration of lidocaine. For standard bronchoscopy, the BF 1T180 videobronchoscope (Olympus Medical Systems Corporation, Tokyo, Japan) was used.

The TBNA biopsy was performed using the MW-122 (22-gauge/13 mm) or SW-121 (21-gauge/15 mm) cytological needles (ConMed Endoscopic Technologies, Utica, New York, United States) or the NA-411D-1521 (21-gauge/15 mm) needle (Olympus Medical Systems Corporation, Tokyo, Japan).

For EBB and TBLB, biopsy forceps (M00515180; working channel diameter, 2.0 mm; jaw outer diameter, 1.8 mm; Boston Scientific, Marlborough, Massachusetts, United States) were utilized. The standard technique of EBB was implemented, with 3 to 8 samples taken from abnormal areas of bronchial mucosa or from the carina of the right upper lobe or the middle lobe. In order to obtain tissue from the deeper part of the mucosa and the submucosal layer of the bronchial wall, several biopsies were taken from the same place.

The site of TBLB was chosen on the basis of the high-resolution chest computed tomography; 2 to 5 samples were taken.

Mediastinal lymph node biopsy was preceded by thorough ultrasound assessment of the entire mediastinum.

For the EBUS-FNA group, a BF-UC160F-OL8 videobronchoscope (Olympus Medical Systems Corporation) with the EU-C60 7.5 MHz ultrasound processor was used. For the biopsy, the cytological 22G/40-mm needle (NA-201SX-4022, Olympus Medical Systems Corporation) was used. Biopsy of lymph node stations 11R, 10R, 11L 10L, 7, 4R, and 4L was performed (Figure 1).

For the EUS-FNA, the GF-UCT160-OL5 videoendoscope (Olympus Medical Systems Corporation) with the EU-C60 7.5 MHz ultrasound processor was used. For biopsy, the cytological 22G/80-mm needle (NA-200H-8022, Olympus Medical Systems Corporation) was utilized. Biopsy of lymph node stations 7 and 4L was performed (Figure 2).

During TBNA, EBUS-FNA, and EUS-FNA, 3 to 6 samples were obtained from each lymph node.
The descriptive statistics, mean and median values, as well as standard deviation and quartiles were used. The sensitivity of tests with a 95% confidence interval was determined using the GraphPad InStat 3.05 software (GraphPad Software, San Diego, California, United States). The bootstrap method was used in Statistica™ (Statsoft Inc., Tulsa, Oklahoma, United States) to compare the sensitivity of different tests. The level of significance was set at a $P$ value of 0.05 or lower.

**RESULTS** Between October 2009 and May 2012, 106 patients were enrolled to the study. There were 61 men and 45 women (mean age, 38.7 years; range, 20–70 years). Stage I sarcoidosis was diagnosed in 55 patients, and stage II, in 51 patients. Coughing and fatigue, which are the most common clinical signs of sarcoidosis, were present in all patients. Ankle swelling was present in 70 patients, and erythema nodosum—in 40.

Having performed the standard diagnostic tests (EBB, TBNA, and TBLB), 55 patients were randomly allotted to group A and 51—to group B. The characteristics of the patients in both groups are presented in Table 1.

In group A, one patient was excluded due to initiation of treatment by his physician before the diagnosis had been confirmed (this was because of signs of neurosarcoidosis and typical clinical picture). In group A, EBUS-FNA failed to establish the diagnosis in 8 patients. In group B, 2 patients were excluded. In one of them, complete remission of mediastinal lymphadenopathy was found on endosonography and in another patient malignant cells were found in the lymph node sample (a diagnosis of lung cancer was subsequently confirmed). In group B, EUS-FNA failed to establish the diagnosis in 8 patients. Therefore, the crossover endosonographic examination was performed in 16 patients. Out of the 8 patients who had undergone the crossover EUS-FNA, the diagnosis was confirmed in 7, while out of the 8 patients who had undergone the crossover EBUS-FNA, the diagnosis was made in 2.

There were no complications related to the procedure or anesthesia in any of the patients.

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**TABLE 1** Characteristics of patients in the study groups

| Characteristics        | Group A (EBUS-NA) | Group B (EUS-NA) | $P$ value |
|------------------------|-------------------|------------------|-----------|
| Age, y, mean (range)   | 38 (20–57)        | 39 (25–70)       | 0.104     |
| Men, n (%)             | 32 (59.3)         | 25 (54.3)        | 0.687     |
| Women, n (%)           | 22 (40.7)         | 21 (45.7)        | 0.687     |
| Stage I sarcoidosis, n (%) | 30 (55.6)      | 24 (52.2)        | 0.841     |
| Stage II sarcoidosis, n (%) | 24 (44.4)      | 22 (47.8)        | 0.841     |
| Steroid therapy, n (%) | 4 (7.4)           | 6 (13)           | 1.000     |

a Data of excluded patients are not included in the table.

Abbreviations: EBUS-FNA, endobronchial ultrasound fine-needle aspiration; EUS-FNA, endoscopic ultrasound fine-needle aspiration.
For the final analysis, data from 100 patients were available. Cytological or histological confirmation of the diagnosis was obtained in all patients. Minimally invasive techniques, that is, EBB, TBNA, TBLB, EBUS-FNA, and EUS-FNA, were successful in 96% of patients, and mediastinoscopy was only necessary in the remaining 4%.

Three patients were excluded at this stage. Two of them withdrew their consent to undergo mediastinoscopy, and in 1 patient complete remission of mediastinal lymphadenopathy was found on endosonography.

Because of the lack of diagnosis, mediastinoscopy was performed in 4 patients and confirmed sarcoidosis in all of them (FIGURE 4).
The sensitivity and accuracy of the standard diagnostic techniques were as follows: 0.12 and 0.12, respectively, for EBB; 0.42 and 0.42, respectively, for TBLB; and 0.42 and 0.44, respectively, for TBNA (Table 2 and Figure 5). A combination of all standard endoscopic techniques (ie, EBB, TBLB, and TBNA) allowed for the confirmation of the diagnosis in 64% of the patients, with sensitivity and accuracy of 0.64.

The diagnostic yield of the endosonographic techniques was higher than that of the standard techniques. For EBUS-FNA, sensitivity and accuracy were 0.62, and for EUS-FNA—0.75. Combination of EBUS-FNA and EUS-FNA had the sensitivity and accuracy of 0.8 (Table 2 and Figure 6).

The bootstrap method was used to compare the sensitivity and accuracy of different tests. Sensitivity and accuracy of each of the endosonographic techniques were significantly better than those of EBB + TBLB ($P = 0.0072$ and $P = 0.0084$, respectively) and than those of EBB + TBLB + TBNA ($P = 0.0112$ and $P = 0.0134$, respectively).

### TABLE 2 Diagnostic yield of bioptic techniques

| Test                  | Sensitivity | 95% CI       | Accuracy | 95% CI       |
|-----------------------|-------------|--------------|----------|--------------|
| EBB                   | 0.12        | 0.06–0.19    | 0.12     | 0.064–0.20   |
| TBLB                  | 0.42        | 0.32–0.52    | 0.42     | 0.32–0.52    |
| TBNA                  | 0.42        | 0.16–0.69    | 0.44     | 0.14–0.69    |
| EBB + TBLB            | 0.44        | 0.34–0.54    | 0.44     | 0.34–0.54    |
| EBB + TBLB + TBNA     | 0.64        | 0.54–0.73    | 0.64     | 0.54–0.73    |
| EBUS-FNA              | 0.62        | 0.49–0.74    | 0.62     | 0.49–0.74    |
| EUS-FNA               | 0.75        | 0.61–0.85    | 0.75     | 0.61–0.85    |
| EBUS-FNA + EUS-FNA    | 0.80        | 0.71–0.87    | 0.80     | 0.71–0.87    |

Abbreviations: EBB, endobronchial biopsy; TBLB, transbronchial lung biopsy; TBNA, transbronchial needle aspiration; others, see Table 1 and Figure 4.
The comparison of EBUS-FNA and EUS-FNA showed higher sensitivity and accuracy of the latter, but the difference did not reach the level of significance ($P = 0.0724$ and $P = 0.0810$, respectively).

The BACTEC examination of bronchial lavage or lymph node samples did not reveal tuberculosis in any of the patients.

**DISCUSSION**  
Cytological or histological confirmation of sarcoidosis is an important element of diagnosis. For this purpose, EBB and TBLB, and also in many centers TBNA, have been used. The widespread use of the endosonographic techniques—EBUS and EUS—for lung cancer staging has given rise to an interest in applying them also to cover the diagnosis of sarcoidosis.  

Our study is novel in that it was based on the assessment of modern, minimally invasive endosonographic techniques and pathological examination of cytological smears rather than histological specimens. The main advantage of the endoscopic techniques is that they save patients from invasive surgical biopsy via mediastinoscopy. Literature data show a trend towards replacement of mediastinoscopy by the minimally invasive techniques. In the present study, the diagnosis of sarcoidosis was confirmed using minimally invasive methods in 96% of the patients, and mediastinoscopy was only necessary in 4%.

An important development in the diagnosis of sarcoidosis is the feasibility of a reliable confirmation on the basis of cytological smear, because it eliminates the need for invasive procedures aimed at taking large samples for a histological examination. An adequately experienced pathologist is crucial, and differentiation with other clinical entities presenting with similar granulomas is necessary. In the present study, all samples were assessed independently by 2 pathologists, and testing for tuberculosis was routinely performed.

It should be noted that, at the time of biopsy, the intensity of symptoms varied among patients. In most of them, biopsy was performed at a point where symptoms had subsided or even almost completely resolved. This was due to the long time of initial diagnostic workup from the first onset of symptoms, before the patients were referred for biopsy. This delay was underlined by Judson et al. It is mainly dependent on clinical symptoms, on the basis of which general practitioners may suspect sarcoidosis. Judson et al. analyzed...
The high sensitivity of endosonographic techniques (71%–100%) has been confirmed by other authors, and these numbers are consistent with the results of the present study.\textsuperscript{2,6,8,14,21} In a randomized study by Tremblay et al.,\textsuperscript{21} the difference in sensitivity between EBUS-FNA and TBNA was 29.5% in favor of the former. In one study, the sensitivity of TBNA was the same as for EBUS-FNA (93%), but the number of patients in this study was very small (n = 15).\textsuperscript{19}

Experience is particularly important in each procedure. In the case of endosonographic techniques, experience of the endoscopic team as well as of pathologists in cytology smear assessment is necessary. In the study of Szlubowski et al.\textsuperscript{21} in a group of 41 patients with suspected sarcoidosis, the diagnosis was confirmed in 85.7% of the cases. In the study of Gnass et al.\textsuperscript{23} (performed with participation of our team), the sensitivity and accuracy of EBUS-FNA were 0.79 and 0.8, respectively, and of EUS-FNA—0.88 and 0.89, respectively. In the study of Szlubowski et al.\textsuperscript{21} the same technique was used, but a different pathology team assessed cytological smears. In the study of Gnass et al.,\textsuperscript{23} as in the present study, patients were diagnosed in the same endoscopy unit. The study of Gnass et al.\textsuperscript{23} was designed and started at the end of enrolling patients to this study. The inclusion and exclusion criteria were similar. Patients in the study of Gnass et al.\textsuperscript{23} were referred from the Departments of Pulmonology at John Paul II Hospital (a different group of patients). Cytological smears were assessed in the same Department of Pathology. The study of Gnass et al.\textsuperscript{23} had a different design but assessed mainly endosonographic techniques. The EBUS/EUS has been used in this endoscopy unit since 2008. During the years from 2009 to 2013 (first study, October 2009 – May 2012, described in this publication; then, January 2012 – February 2013, described in the study of Gnass et al.\textsuperscript{23}), the endoscopic and pathology teams gained more experience in the technique, which resulted in much higher sensitivity of the procedures.

In a prospective study by Tournoy et al.,\textsuperscript{26} endosonographic techniques confirmed the diagnosis of sarcoidosis in an additional 59% of patients in whom the results of the standard endoscopic techniques had been negative.

The standard endoscopic techniques (i.e., EBB, TBLB, and TBNA) were compared with EBUS-FNA by Navani et al..\textsuperscript{24} The sensitivity of these methods were 35% and 85%, respectively. Von Bartheld et al.\textsuperscript{25} conducted a prospective study including 101 patients with suspected sarcoidosis in whom standard endoscopic techniques had failed to confirm the diagnosis in 55%. The sensitivity of EUS-FNA was 92% in stage I and 77% in stage II sarcoidosis. The authors suggested that 4 needle passes are optimal for a reliable diagnosis.\textsuperscript{18}

In the present study, EBB enabled the confirmation of diagnosis only in 12% of the patients. It has been reported that its diagnostic yield is higher if there are bronchoscopically detectable lesions of the bronchial mucosa. Armstrong et al.,\textsuperscript{25} in a group of 101 patients with suspected sarcoidosis, found sensitivity of EBB to be 58% in stage I and 62% in stage II sarcoidosis. The authors also noted that the rate of diagnosis was 91% if there were mucosal lesions, but only 37% in case of normal mucosa. Sensitivity similar to the present study (0.14) was reported by Ziora et al.\textsuperscript{26} in a study which included 112 patients.
The results of the present study confirm the superiority of EBUS-FNA and EUS-FNA in the diagnosis of stage I and II sarcoidosis. However, the availability of adequately equipped endoscopy suites and staff trained in both EBUS and EUS is a factor limiting the widespread use of these techniques. In particular, pulmonologists generally do not have experience in esophageal endoscopy, and gastroenterologists do not perform bronchoscopy. At present, endoscopists experienced in EBUS and EUS are only available in a few institutions.

Therefore, in institutions with limited access to EBUS and EUS, the routine use of TBNA seems a reasonable option. In the present study, the sensitivity of TBNA was 0.44, which is comparable to literature data reporting a sensitivity of TBNA between 0.38 and 0.90.\textsuperscript{5,7,12,27} In a meta-analysis of 21 studies encompassing 915 patients, the mean sensitivity of TBNA was 0.62 (CI, 0.52–0.71).\textsuperscript{15}

The diagnostic yield of TBNA depends on several factors, such as the gauge on the needle, the size and location of the nodes, and the endoscopist’s experience. In a study including 100 patients, Herth et al\textsuperscript{28} compared EBUS-FNA and TBNA in the assessment of different nodal stations in patients with adenopathy of different origin. The authors found that for stations other than subcarinal, EBUS-FNA was superior to TBNA, with a diagnostic yield of 0.84 and 0.58, respectively ($P < 0.001$). The respective values for the subcarinal nodes were 0.86 and 0.74, and the difference was not significant ($P = 0.3$).\textsuperscript{28}

It is likely that remission of lymphadenopathy contributed to the lower sensitivity of TBNA in the present study. If the TBNA had been performed at an early stage of disease, with marked lymph node enlargement, the sensitivity would have probably been higher. It should be stressed that during TBNA, 3 to 6 samples were obtained from each lymph node, which is in accordance with the published standard.\textsuperscript{28}

The sensitivity of TBLB in the present study was 0.42, which is consistent with literature data, reporting a sensitivity of 0.35 to 0.90.\textsuperscript{21-23,29-31}

Descombes et al\textsuperscript{32} analyzed 530 TBLB in 516 patients and reported a sensitivity of 0.56 in stage I and 0.75 in stage II sarcoidosis. If 6 to 10 samples were obtained, the diagnosis was established in 69% of patients, while it was 38% if the number of samples was between 1 and 3. This difference was significant ($P < 0.01$).\textsuperscript{32} In the present study TBLB confirmed sarcoidosis in 19 patients with normal plain chest radiographs. These figures are also consistent with literature data.\textsuperscript{21}

The results of the present study showed higher sensitivity and accuracy of EUS-FNA than of EBUS-FNA, but the difference was not significant. There were no complications in any of the groups.

In conclusion, the diagnostic sensitivity and accuracy of endoscopic techniques (EBUS-FNA and EUS-FNA) are significantly higher in comparison with the standard endoscopic methods ($P < 0.01$). Endoscopic techniques seem to be the methods of choice in patients suspected of sarcoidosis stages I and II. A combination of standard endoscopic methods, especially TBNA and TBLB, increases the yield of confirmation of sarcoidosis.

### Contribution statement
PK, JK, and AS conceived the idea for the study and contributed to the design of the research. PK, AS, JW, JRW, PT, TS, TG, JW, and LR-S were involved in data collection. PK and AC analyzed the data. LR-S and RT performed pathological assessment of all samples. PK, JK, AS, and JS edited the manuscript. All authors approved the final version of the manuscript.

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