Endoscopic ultrasound-guided fine-needle aspiration for the diagnosis of sarcoidosis

Background and study aims: Transesophageal endoscopic ultrasound-guided fine-needle aspiration (EUS-FNA) of mediastinal lymph nodes is increasingly used to detect noncaseating granulomas in patients with suspected sarcoidosis. The optimal needle size and tissue processing method for detecting noncaseating granulomas are debated. We assessed the value of cell-block analysis when added to conventional cytological evaluation of EUS aspirates obtained by 22-gauge needles in patients with stage I and II sarcoidosis.

Patients and methods: Data from 101 consecutive patients (55% of whom had previously had a nondiagnostic bronchoscopy) with suspected pulmonary sarcoidosis (stage I and II), who underwent EUS-FNA of mediastinal lymph nodes with 22-gauge needles were retrospectively analyzed.

Results: The sensitivity of EUS in detecting granulomas was 87% (cytology and cell-block analysis together) (stage I, 92%; stage II, 77%). In 33% of cytology negative patients (n = 6), granulomas were present in the cell block. The optimal yield for granuloma detection was reached with four needle passes. One patient developed mediastinitis after EUS-FNA.

Conclusions: Cell-block analysis added to conventional cytological evaluation of 22-gauge EUS aspirates, results in a high yield in detecting granulomas in patients with suspected sarcoidosis and reduces the false-negative rate substantially. EUS has a considerably higher yield in stage I compared with stage II sarcoidosis. For an optimal yield, four needle passes are required.
In addition, we assessed the optimal number of needle passes and the difference in yield between stage I and II sarcoidosis.

Patients and methods

Patients

At our hospital, EUS-FNA is often used as the diagnostic test of choice to confirm noncaseating granulomas in patients with suspected sarcoidosis. In the present study, data from all consecutive patients who were scheduled, between 2004 and 2009, to undergo EUS-FNA for a tissue confirmation of noncaseating granulomas were retrospectively analyzed.

Of these 101 patients (54% men, 46% women; mean age 44 years, range 22–79) with suspected sarcoidosis stage I (54%) or stage II (46%), 56 patients (55%) had previously undergone a nondiagnostic fiberbronchoscopy in which the following interventions were performed: TBNA, n = 21; transbronchial needle aspiration (TBNA), n = 18; endobronchial biopsy (EBB), n = 13; BAL, n = 29.

Handling of EUS aspirates

EUS aspirates were expelled from the needle either by blowing air through a 50-ml syringe or by reinserterion of the stylet. Aspirates were smeared on glass slides and a proportion of slides were taken for on-site evaluation and colored with Diff-Quick staining (Kit RAL-555; Reactifs RAL, Martillac, France) for adequacy assessment by the EUS investigators (J.T.A. or K.F.R.) during the EUS procedure. After the procedure, all slides were sent for interpretation by an experienced cytopathologist (M.V.C.) and the EUS investigators (J.T.A. or K.F.R.) during the EUS procedure. All statistical calculations were done with SPSS version 16.0 (SPSS Inc. Chicago, Illinois, USA).

EUS-FNA

All EUS-FNA procedures were performed on an outpatient basis in the Leiden University Medical Center, a tertiary referral center for EUS-FNA, by two experienced pulmonologists (K.F.R., J.T.A.). Investigations were done with patients under conscious sedation with midazolam (1 to 5 mg intravenously) and were performed using a linear Pentax FG-34UX echo endoscope in combination with a Hitachi EUB-6500 ultrasound scanner. A standardized examination of all mediastinal nodal regions that can be detected from the esophagus was carried out and ultrasound characteristics of the mediastinal lymph nodes were described (Fig. 1).

In patients in whom mediastinal tuberculosis was also considered aspirates were sent for polymerase chain reaction (PCR) analysis and culture of Mycobacterium tuberculosis.

Final diagnosis and data analysis

The final diagnosis of sarcoidosis was based on clinical and radiological suspicion, tissue confirmation of noncaseating granulomas, and a follow-up period after which similarly presenting diseases such as lung cancer, lymphoma or tuberculosis could be excluded.

Lymph node aspirates obtained by EUS-FNA were considered to be representative if noncaseating granulomas or nodal lymphoid tissue were found, or if the aspirate contained other cellular material that resulted in a specific diagnosis.

The sensitivity of EUS-FNA for the assessment of noncaseating granulomas was calculated as the number of patients in whom EUS demonstrated granulomas as a proportion of the total of patients with a final diagnosis of sarcoidosis.

To find the optimal number of needle passes, the cumulative sensitivity for successive needle passes was calculated as follows: the number of patients in whom a particular needle pass gave the first confirmation of granulomas was added to the total already having an EUS confirmation, and this new total was divided by the total number of patients with a final diagnosis of sarcoidosis.

All statistical calculations were done with SPSS version 16.0 (SPSS Inc. Chicago, Illinois, USA).

Results

EUS-FNA

Standardized EUS examination of the mediastinal nodes was feasible in 100 patients (99%) (Fig. 2), while introduction of the EUS scope in the esophagus was not possible in a single patient because of extreme sensitivity of the pharynx.
Aspirates of mediastinal lymph nodes were obtained in all patients, with an average of 3.9 needle passes (range 1–7) per patient. Aspirated lymph nodes were located in the paratracheal areas (stations 2R, 4L, 4R) in 21 patients (21%), in the aortopulmonary window (station 5) in 5 patients (5%), the subcarinal area (station 7) in 97 patients (97%) and in the lower paraesophageal region (stations 8L, 8R) in 9 patients (9%).

Cytological smear examinations were done for all 100 patients and cell-block analysis was available for 76 (76%). Cytological smears showed noncaseating granulomas without necrosis in 72 patients (Fig. 3a), noncaseating granulomas with necrosis in 1, reactive lymph node tissue with loose epithelioid cells in 4, reactive nodal tissue with giant cells in 1, non-small-cell lung carcinoma in 2, Reed–Sternberg cells in 1, and reactive nodal tissue in 11, and the aspirate from 8 patients contained nonrepresentative material.

Cell blocks of the EUS aspirates demonstrated noncaseating granulomas without necrosis in 72 patients, noncaseating granulomas with necrosis in 1, reactive lymph node tissue with loose epithelioid cells in 4, reactive nodal tissue with giant cells in 1, non-small-cell lung carcinoma in 2, Reed–Sternberg cells in 1, and reactive nodal tissue in 11 and the aspirate from 8 patients contained nonrepresentative material.

Cytology and cell-block investigation together detected noncaseating granulomas in 79 patients. In 18 patients without noncaseating granulomas in cytological smears, cell-block analysis confirmed granulomas in 6 (33%). Tissue that was representative of mediastinal lymph nodes was obtained for 95 patients (95%). Culture and PCR analysis for M. tuberculosis was done for 28 patients (28%) and were negative for all.

Further diagnoses and follow-up
Among the 21 patients without granulomas at endoscopic ultrasound, EUS-FNA found non-small-cell lung carcinoma in two and non-Hodgkin lymphoma (Reed–Sternberg cells) in one. In five patients, granulomas were subsequently found, by mediastinoscopy (n = 3), bronchoscopy with transbronchial biopsy (n = 1), and by nodal excision in the groin (n = 1). One patient was diagnosed by means of bone biopsy with metastasized adenocarcinoma of unknown origin, one patient with malignant lymphoma by mediastinoscopy, and in one patient PCR analysis was positive for atypical mycobacteria. The ten remaining patients were clinically and radiologically followed. For the whole cohort, follow-up was available for a median period of 18 months (range 1–53) and follow-up for a period of at least 6 months was available for 90 patients (90%).

Final diagnosis
A final diagnosis of sarcoidosis was made in 91 patients (91%). The other diagnoses were non-small-cell lung carcinoma in three (3%), malignant lymphoma in two (2%), atypical mycobacterial infection in one (1%), extrinsic allergic alveolitis in one (1%) and reactive lymphadenitis in two (2%). Based on cytological smears and cell-block analysis together, EUS-FNA detected noncaseating granulomas in 79 patients (87%).
Table 1  The cumulative sensitivity of endoscopic ultrasound-guided fine-needle aspiration (EUS-FNA) for the detection of noncaseating granulomas with consecutive needle passes, in 91 patients with a final diagnosis of sarcoidosis.

<table>
<thead>
<tr>
<th>Needle passes (FNA)</th>
<th>Patients receiving this needle pass, n</th>
<th>Patients in whom this needle pass demonstrated granulomas for the first time, n</th>
<th>Cumulative sensitivity in detecting granulomas</th>
</tr>
</thead>
<tbody>
<tr>
<td>1st</td>
<td>91</td>
<td>50</td>
<td>55%</td>
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<tr>
<td>2nd</td>
<td>91</td>
<td>20</td>
<td>77%</td>
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<td>3rd</td>
<td>79</td>
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<td>80%</td>
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<td>4th</td>
<td>54</td>
<td>5</td>
<td>86%</td>
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<tr>
<td>5th</td>
<td>26</td>
<td>0</td>
<td>86%</td>
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<tr>
<td>6th</td>
<td>9</td>
<td>0</td>
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<tr>
<td>7th</td>
<td>4</td>
<td>1</td>
<td>87%</td>
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The sensitivity for stage I sarcoidosis was 92% (n = 52), whereas it was 77% for stage II (n = 39).
The sensitivity of EUS-FNA for the detection of granulomas in patients without a previous bronchoscopy (n = 40), a previous bronchoscopy with tissue sampling (TBLB, EBB, TBNB) (n = 43) and a previous bronchoscopy with only BAL (n = 8) was 93%, 84% and 63%, respectively.
The cumulative sensitivity of EUS-FNA for the confirmation of noncaseating granulomas with one, two, three, and four needle passes was 55%, 77%, 80%, and 86%, respectively. Additional needle passes after the fourth one did not notably increase the diagnostic yield (Table 1).

In patients with a final diagnosis of sarcoidosis, EUS features suggestive for sarcoid involvement (multiple, clustered isoechoic lymph nodes) were noted in 79 patients (87%). In the other 12 patients, lymph nodes were either small and non-suspicious (< 10 mm, oval-shaped, vaguely demarcated borders) (n = 5), consisted of only a solitary, isoechoic enlarged lymph node (n = 5), or presented with inhomogeneous echo features (n = 2).

Safety
One serious complication (1%) occurred in a 49-year-old woman with a final diagnosis of sarcoidosis who developed fever and retrosternal pain, 2 days after EUS-FNA of subcarinal and lower paraesophageal lymph nodes in which noncaseating granulomas were found. She reported to the hospital 10 days after developing symptoms, and computed tomography (CT) images showed local abscesses in the biopsied mediastinal area. During thoracotomy, necrotic lymph node tissue was removed after which the patient recovered completely.
Minor adverse effects were recorded in two other patients with a final diagnosis of sarcoidosis (2%). In one patient a small local hematoma was seen on ultrasound images directly after FNA of a subcarinal lymph node and another patient complained of painful swallowing following EUS.

Discussion
In patients with suspected sarcoidosis, EUS-FNA with 22-gauge needles demonstrated noncaseating granulomas in 87% (n = 79) by aspiration of mediastinal lymph nodes (conventional cytology plus cell-block investigation). The addition of cell-block analysis to cytological smear examination of EUS fine-needle aspirates reduced the false-negative rate by 33%. The yield of EUS-FNA for the detection of noncaseating granulomas was optimal when four needle passes were performed. The diagnostic accuracy for radiological stage I sarcoidosis was 92%, whereas it was 77% for stage II disease.
The observed high yield in assessing noncaseating granulomas in the present cohort of patients with a suspicion of sarcoidosis confirms the findings of other smaller studies.
In our previous published paper, reporting on another 51 patients, we found a diagnostic accuracy of 82% based on cytology without cell-block analysis [8]. Other studies have reported sensitivities of 86%–100% in patients with sarcoidosis [9–12]. In the present study, six potential false-negative EUS-FNA outcomes were prevented because cell-block analysis demonstrated granulomas whereas conventional cytological smears did not. This additional value of the cell-block investigation may be explained by the way the cell block is processed for diagnostic analysis. Noncaseating granulomas are characterized by groups of epithelioid cells and the structure of these cells could be disrupted when the aspirates are smeared between two glass slides. Material obtained by FNA and subsequently collected in a paraffin-embedding container is not liable to this disruption of the anatomical structure.
Regarding granuloma detection, Iwashita et al. demonstrated an added value of histological examination (94%) over cytological smear (78%) in 41 patients with stage I sarcoidosis, using a 19-gauge needle [11]. In the present study, a similar sensitivity for stage I sarcoidosis (92%) was obtained in 52 patients with 22-gauge needles when cytological smear and cell-block analysis of fine-needle aspirates were combined.
When comparing our study with that of Iwashita et al., some practical differences regarding the handling of the material should be noted. In the study of Iwashita et al., “whitish” samples were handpicked from the glass slides and subsequently separated from the rest of the fine needle aspirate. In the present study, the aspirates for cell-block evaluation were simply sprayed into a formalin tube. Collecting the major part of the aspirates in a tube for cell-block analysis also avoids a large number of glasses being sent to the pathologist, since nodal aspirates of patients with sarcoidosis are often bloody, and processing them all on glass slides greatly increases the workload for cytopathologists.
In the present study, we demonstrated that with four needle passes, an optimal yield can be obtained for the detection of granulomas. Therefore, at least four needle passes are advised in the absence of on-site cytology.
Although the complication rate with 19-gauge needles seems to be similar to that with 22-gauge needles, it has been reported that 19-gauge needles are stiffer and less maneuverable compared with 22-gauge needles [13].
The diagnostic accuracy of EUS-FNA for noncaseating granulomas was significantly higher for stage I (92%) than for stage II
been shown to obtain yields similar to those of the present study and include endobronchial ultrasound-guided transbronchial needle aspiration (EBUS-TBNA), TBLB, TBNA, and EBB. EBUS-TBNA has been shown to obtain yields similar to those of the present study for the confirmation of noncaseating granulomas (range 85% – 96%) [14 – 16]. To date, no study has been done that has compared the value of EUS-FNA versus EBUS-TBNA in patients with suspected sarcoidosis.

With TBLB, described by the 1999 WASOG/ERS/ATS consensus statement as the advised diagnostic method, approximately one third of patients remain non-diagnostic [5,6] and the technique is associated with a 7% risk of pneumothorax or bleeding (> 50 ml) [7]. The yield of transbronchial needle aspiration (TBNA) is also modest, at just over 60% [17,18]. Although it has been advocated that a bronchoalveolar lavage (BAL) with an increased CD4/CD8 ratio is very specific for the diagnosis of sarcoidosis [19], several studies have reported highly variable results [20 – 22].

In the present study, one serious complication was observed when a lymphadenitis with abscess formation developed after EUS-FNA. Whether treatment with antibiotics, directly after the onset of symptoms would have altered the course of infection is unknown. In the study of Iwashita et al., one patient also developed a mediastinitis after EUS-FNA [11]. Several limitations apply to this study. First of all, only patients with stage I and II sarcoidosis were included in this analysis and therefore the data only apply to these stages. Secondly, the prevalence of tuberculosis in our geographical area is low and other granulomatous diseases such as histoplasmosis or berylliosis are also rarely seen. Therefore, in terms of external validity, this study is only applicable to regions where these diseases are similarly prevalent. Also, PCR analysis and culture for M. tuberculosis was not performed for every patient.

Furthermore, the study setting is a tertiary referral centre and patients with suspected sarcoidosis are mostly referred for a tissue confirmation of sarcoidosis because they present with active symptoms, which might represent a patient group in which there is a high chance of finding noncaseating granulomas. Finally, material for cell-block evaluation was not obtained in every patient. Cell-block analysis was performed in the absence of granulomas in the first smear during on-site evaluation, or when the acquired material was abundant or very bloody. The latter may explain why no representative material was found in one third of the cell blocks.

In conclusion, EUS-FNA with 22-gauge needles is a feasible, safe, and highly diagnostic technique for the tissue confirmation of noncaseating granulomas in patients with suspected sarcoidosis. Regarding granuloma detection, cell-block analysis added to conventional cytological smears reduces the false-negative rate considerably, and should therefore be used routinely in all patients who undergo EUS-FNA for the diagnosis of sarcoidosis. EUS has a substantially higher yield for stage I compared with stage II sarcoidosis. For an optimal yield in the detection of granulomas, a minimum of four needle passes are advised in the absence of on-site cytological evaluation. A randomized, prospective study between ultrasound-guided fine-needle aspiration of mediastinal or hilar lymph nodes (by either EUS-FNA or EBUS-TBNA) and transbronchial biopsies for the assessment of noncaseating granulomas is indicated, to investigate the optimal diagnostic strategy for patients suspected of pulmonary sarcoidosis.

Competing interests: None

References