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Esophageal Endoscopic Ultrasound With Fine-Needle Aspiration With an On-site Cytopathologist*

High Accuracy for the Diagnosis of Mediastinal Lymphadenopathy

Kurt G. Tournoy, PhD; Marleen M. Praet, PhD; Georges Van Maele, PhD; and Jan P. Van Meerbeeck, PhD

Study objectives: To analyze the accuracy of esophageal endoscopic ultrasound (EUS) with real-time, guided fine-needle aspiration (EUS-FNA) with an on-site cytopathologist in patients with (presumed) lung cancer presenting with mediastinal lymphadenopathy (ML) or a suspect left adrenal gland (LAG).

Design: A single-center prospective study.

Patients: Sixty-seven outpatients with (presumed) lung cancer with ML or a suspect LAG on either CT and/or positron emission tomography with 18F-fluorodeoxyglucose (FDG-PET) scan.

Interventions: All patients underwent EUS-FNA under conscious sedation. A cytopathologist was present during all procedures.

Measurements: EUS with and without fine-needle aspiration (FNA) as compared to FDG-PET was evaluated for accuracy in diagnosing cancer, safety, and rate of avoidance for further surgery.

Results: Of 67 consecutive patients (56 men; median age, 64 years), malignant ML or LAG were found in 47 patients (70.1%). In 20 patients (29.9%) without EUS-FNA proof of malignancy, confirmation was obtained by surgical procedure in 13 patients (sarcoïdosis [n = 5], infection [n = 1], lung cancer [n = 7]) or by clinical follow-up in 5 patients suggesting benign disease.

Sixty-five patients were included in the calculation of test characteristics. With malignancy as an end point, the accuracy for EUS-FNA was 100%. This was better than EUS without FNA (accuracy, 75.4%; p < 0.001) or FDG-PET (accuracy, 75.0% [n = 28]; p = 0.0011). When using final histopathologic diagnosis as an end point, the accuracy of EUS-FNA was 92.3%, since EUS-FNA was unable to show noncaseating granulomas in those patients with sarcoïdosis diagnosed after mediastinoscopy. Related to the presence of the in situ cytopathologist, there were no inconclusive samples. No adverse events were recorded, and 67.7% of surgical interventions were avoided following EUS-FNA.

Conclusions: The accuracy in this series of EUS-FNA with cytopathologist-assisted rapid on-site evaluation is high. The technique is safe and greatly reduces the number of surgical interventions.

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Key words: endoscopic ultrasound; fine-needle aspiration; lung cancer; mediastinum; rapid on-site evaluation

Abbreviations: EUS = esophageal endoscopic ultrasound; EUS-FNA = esophageal endoscopic ultrasound with real-time, guided fine-needle aspiration; FDG-PET = positron emission tomography with 18F-fluorodeoxyglucose; FNA = fine-needle aspiration; LAG = left adrenal gland; ML = mediastinal lymphadenopathy; ROSE = rapid on-site evaluation; TBNA = transbronchial needle aspiration

The implementation of new diagnostic tools in routine medical practice follows extensive preclinical and clinical testing. Curved linear esophageal endoscopic ultrasound (EUS) with real-time guided fine-needle aspiration (EUS-FNA) is a fairly new tool for chest physicians in the diagnostic approach of mediastinal lymphadenopathy (ML).1,2

Diagnosis of ML in patients with presumed lung cancer remains a challenge since treatment and prognosis are dictated by tumor stage. The accuracy
of the CT scan to predict ML tumor invasion is limited.\(^3\) Positron emission tomography with \(^{18}\text{F}\)-fluorodeoxyglucose (FDG-PET) is more accurate for evaluating the mediastinum.\(^4\) However, although FDG-PET has a good negative predictive value (> 93%), it has a low specificity.\(^5\) Therefore, the current recommendation is to obtain pathologic confirmation of ML in patients with (presumed) lung cancer in whom CT or FDG-PET show ML. A surgical procedure (mediastinoscopy, thoracoscopy, or thoracotomy) is considered the “gold standard.”\(^6\) Transbronchial needle aspiration (TBNA) is advocated by some specialized groups.\(^7\) However, TBNA does not have real-time puncture guidance, and its accuracy is highly operator dependent. Endobronchial ultrasound with real-time guided puncture is a new technique for staging of the mediastinum. The technique is promising but still experimental.\(^1,8\)

The combined use of radial and linear EUS for investigation of mediastinal structures is well established in gastrointestinal. In contrast with radial EUS, which is characterized by high-frequency (20 MHz), detailed images but which does not allow fine-needle aspiration (FNA), linear EUS-FNA (5 to 10 MHz) has a deeper tissue penetration. Hence, it allows real-time guided FNA but is unable to discriminate mediastinal pleural layers. Linear EUS-FNA therefore needs to be used with caution for making decisions about direct mediastinal invasion through the pleural layers.\(^9\) Nonrandomized trials\(^10-15\) report encouraging results of EUS techniques for the staging of (presumed) lung cancer with a good specificity but variable sensitivity (0.72 to 0.97) in analyzing ML. However, most of these reports\(^10-13\) were performed with the sequential use of radial and linear EUS. Few reports\(^14,15\) studied the role of the linear EUS-FNA only, and were performed in selected patients with FDG-PET-positive ML.

Rapid on-site evaluation (ROSE) is comparable with intraoperative frozen-section examination and requires at least the presence of a laboratory assistant in order to process the freshly obtained material and to report to the endoscopist whether the obtained material is adequate for diagnosis. Although ROSE has been shown to improve the diagnostic yield of endoscopic procedures, there is still a problem of high numbers of inconclusive smear results.\(^14\) Several investigators\(^14\) hinted at but never assessed the potential benefit of an on-site cytopathologist. We report the accuracy of esophageal EUS-FNA with cytopathologist-assisted ROSE in consecutive patients with ML or a suspect left adrenal gland (LAG).

### Materials and Methods

#### Patients

Consecutive patients presenting in a pulmonary department with suspect ML\(^16\) at levels 2, 4, 5, 7, 8, and 9, or a suspect LAG were prospectively investigated with EUS-FNA. ML or the LAG was considered suspect when the transversal diameter was > 10 mm on CT scan or if it was FDG positive on the positron emission tomography scan. All patients would routinely have been scheduled for further diagnostic procedures, either by mediastinoscopy, thoracoscopy, thoracotomy, or adrenal puncture.

#### Procedure and Design

A curved linear scanning ultrasound endoscope (GF-UCT180-OL5; Olympus; Aartselaar, Belgium) connected to an ultrasonic unit (ALOKA; Mechelen, Belgium) was used in this series. The investigation was performed by a trained operator (K.G.T.) in an outpatient setting with a patient under conscious sedation (IV midazolam, 1 mg, until convenient for patient and investigator) while monitoring the pulse rate and peripheral oxygen saturation.\(^17\) The endoscope was guided into the stomach to scan the LAG area and the area around the celiac trunk. After retraction into the esophagus, the mediastinum was evaluated by scanning 360° transaxially at intervals of approximately 1 cm upward to ML level 2. The MLs were identified as nonpulsating regions with round, ellipsoid, crescent, or triangular shapes. All MLs measuring > 3 mm on one of the axes were considered. These were considered suspect if one of the following characteristics were present: hypoechoegenecity, sharp edges, round shape, and largest diameter > 10 mm. Absence of major blood vessels was confirmed by Doppler flow in the region of interest. The largest diameter of the ML was measured. All punctures were performed with a 22-gauge fine needle (EUS-needle; Olympus; Aartselaar, Belgium). Only one needle per procedure was used. Suspect MLs ipsilateral to the lung lesion were only punctured if contralateral MLs were either considered not suspect or negative at puncture.

In those patients with positive ML or LAG findings after EUS-FNA, no additional confirmation of malignancy was done. We assumed that none of the biopsy results were false-positive since care was taken not to puncture primary tumors. A surgical intervention was proposed in those patients with ML but negative EUS-FNA findings. In some cases, an extended clinical/radiologic follow-up documenting absence of disease progression was considered as a proof for benign underlying disease.

#### Sample Processing

Smears of the aspirates obtained by EUS-FNA were processed in situ. For ROSE, the cytotechnician and cytopathologist (M.M.P.) were called once the first puncture was initiated. They

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*From the Department of Pulmonary Diseases, Endoscopy Unit (Drs. Tournoy, Van Maelle, and Van Meerbeeck), and N. Goormaghtigh Institute of Pathology (Dr. Praet), Ghent University Hospital, Ghent, Belgium.

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Correspondence to: Kurt G. Tournoy, PhD, Department of Pulmonary Diseases, Endoscopy Unit, Ghent University Hospital, De Fintelaan 185, 9000 Ghent, Belgium; e-mail: kurt.tournoy@UGent.be
evaluated the cellular contents of the air-dried specimens on-site with a quick staining method (Diff-Quick; Medicon Diagnostics; Dortmund, Germany). If a specimen was considered inadequate, additional punctures were performed with the total number of punctures being noted. If necessary, several lymph nodes were sampled. Specimens were categorized as positive (tumor cells), negative (lymphoid but no tumor cells), or inconclusive (poor cellularity). Punctures were continued until the cytopathologist was able to make a formal conclusion. Depending on the amount of material, additional Papanicolaou (with previous short fixation) and Giemsa staining were performed.

**Statistical Analysis**

All categorical variables are reported as proportions. Continuous variables are reported as medians and minimum-maximum range. Comparison of proportions (accuracy analysis) was done with a Z test. A two-sided \( p < 0.05 \) was considered statistically significant. The analysis was done using statistical software (SPSS 12.0; SPSS; Chicago, IL).

**Results**

**Patients and Procedure Characteristics**

Between March 2004 and March 2005, 67 consecutive patients with suspect ML or LAG were registered. Demographic and procedural characteristics are shown in Table 1.

Referral for EUS-FNA was for staging reasons in 30 patients (44.8%) in whom proof of non-small cell lung cancer was already obtained. Thirty-seven patients (55.2%) had suspect ML or LAG without formal proof. FDG-PET data were available in 28 patients (41.8%).

With EUS, we visualized suspect ML or LAG in one station in 24 patients (35.8%), in two stations in 20 patients (29.9%), in three stations in 16 patients (23.9%), and in four to five stations in 7 patients (10.5%). Subcarinal ML was most frequently detected (89.6% of the patients), followed by the closely adjacent regions 4L and 5 (together, 73.2%). EUS indicated a suspect LAG in six patients (9.0%). The primary tumor was observed in 17.9% but was never punctured. A median of three punctures (range, one to eight punctures) was performed per patient.

The median use of midazolam was 3.0 mg per procedure, and the median duration of the procedure was 45 min. No complications or adverse events were reported.

**Table 1—Demographic Characteristics of the Patients and EUS-FNA Procedure Characteristics**

<table>
<thead>
<tr>
<th>Variables</th>
<th>Data</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patients, No.</td>
<td>67</td>
</tr>
<tr>
<td>Median age, yr (range)</td>
<td>64 (27–81)</td>
</tr>
<tr>
<td>Gender</td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>56 (83.6)</td>
</tr>
<tr>
<td>Female</td>
<td>11 (16.4)</td>
</tr>
<tr>
<td>Aim of the procedure</td>
<td></td>
</tr>
<tr>
<td>Diagnostic</td>
<td>37 (55.2)</td>
</tr>
<tr>
<td>Staging only (known malignancy)</td>
<td>30 (44.8)</td>
</tr>
<tr>
<td>Position of lymph nodes detected on EUS (( \geq 3 ) mm)</td>
<td></td>
</tr>
<tr>
<td>2R</td>
<td>2 (3.0)</td>
</tr>
<tr>
<td>2L</td>
<td>1 (1.5)</td>
</tr>
<tr>
<td>4R</td>
<td>1 (1.5)</td>
</tr>
<tr>
<td>4L</td>
<td>20 (29.9)</td>
</tr>
<tr>
<td>5</td>
<td>29 (43.3)</td>
</tr>
<tr>
<td>7</td>
<td>60 (89.6)</td>
</tr>
<tr>
<td>8R</td>
<td>8 (11.9)</td>
</tr>
<tr>
<td>8L</td>
<td>12 (17.9)</td>
</tr>
<tr>
<td>9R</td>
<td>3 (4.5)</td>
</tr>
<tr>
<td>9L</td>
<td>3 (4.5)</td>
</tr>
<tr>
<td>LAG</td>
<td>6 (9.0)</td>
</tr>
<tr>
<td>EUS visualization of primary tumor</td>
<td>12 (17.9)</td>
</tr>
<tr>
<td>Median duration of the procedure, min (range)</td>
<td>45 (20–75)</td>
</tr>
<tr>
<td>Median midazolam dose, mg (range)</td>
<td>3.0 (0.0–7.5)</td>
</tr>
<tr>
<td>Median of largest lymph node axis, mm (range)</td>
<td>23 (4–75)</td>
</tr>
<tr>
<td>Median EUS passes, No. (range)</td>
<td>3 (1–8)</td>
</tr>
</tbody>
</table>

*Data are presented as No. (%) unless otherwise indicated.

30 patients (44.8%) in whom proof of non-small cell lung cancer was already obtained. Thirty-seven patients (55.2%) had suspect ML or LAG without formal proof. FDG-PET data were available in 28 patients (41.8%).

With EUS, we visualized suspect ML or LAG in one station in 24 patients (35.8%), in two stations in 20 patients (29.9%), in three stations in 16 patients (23.9%), and in four to five stations in 7 patients (10.5%). Subcarinal ML was most frequently detected (89.6% of the patients), followed by the closely adjacent regions 4L and 5 (together, 73.2%). EUS indicated a suspect LAG in six patients (9.0%). The primary tumor was observed in 17.9% but was never punctured. A median of three punctures (range, one to eight punctures) was performed per patient.

The median use of midazolam was 3.0 mg per procedure, and the median duration of the procedure was 45 min. No complications or adverse events were reported.

Results of EUS-FNA

The outcome of this series of patients is shown in Figure 1. In 20 patients (29.9%), no malignancy was found with EUS-FNA. Of this latter group, 13 (19.4%) underwent a surgical procedure. By means of mediastinoscopy, granulomatous disease suggestive for sarcoidosis was diagnosed in five patients. Of the seven operated lung cancer patients, all had a pN0–1 stage. Nonspecific inflammatory changes were diagnosed in one patient that post hoc were explained in the context of tuberculosis. In five patients in whom no surgical procedure was performed, the clinical follow-up of at least 5 months suggested a nonmalignant cause of ML. There were two patients (3.0%) with negative EUS-FNA findings in whom no confirmation about the nature of the ML was obtained because of patient-related reasons.

In 47 patients (70.1%), a malignant ML or LAG were detected with EUS-FNA. When excluding the five patients with LAG involvement, 67.7% of the patients in whom a surgical intervention to stage the mediastinum was planned were not referred to the surgeon. Regions 5, 8R, 8L, 9R, 9L, and the LAG are targets that are out of reach for cervical mediastinoscopy. These were assessed by EUS-FNA in 27 patients (40.3%) and showed malignancy in 19 patients (70.4%).

Table 2 shows the final diagnosis of the ML obtained after all procedures. The characteristics of the tumor obtained with EUS-FNA in the 47 patients were compatible with non-small cell lung cancer in 42 patients. All three main subtypes of
non-small cell lung cancer were present in this series, with the subtype of epidermoid epithelioma being most frequently detected. In three patients, we found small cell lung cancer, while two cases of metastasis of other cancers were found (one renal cell carcinoma and one bladder carcinoma).

In 53 patients in whom only one station was punctured, proof of malignancy was found in 43 patients (81.1%). In 14 patients in whom more than one station was punctured, malignancy was shown in only 4 patients (28.6%), indicating that the finding of one negative lymph node station with EUS-FNA does not preclude malignant invasion of another station.

Operating Characteristics of EUS and EUS-FNA as Compared With FDG-PET

The operating characteristics for EUS and EUS-FNA were calculated for the 65 patients in whom a final diagnosis was obtained (Table 3). When taking malignancy as an end point, the accuracy of EUS without FNA is similar to the accuracy of the FDG-PET scan (75.0% vs 75.4%, p = not significant). EUS-FNA has an accuracy of 100% as compared to both EUS without FNA (p < 0.001) and FDG-PET (p = 0.0011). When taking the final diagnosis as an end point, the accuracy of EUS-FNA is 92.3%.

Table 2—Final Pathology Diagnosis of the Mediastinal Lymph Nodes in the Study Population

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>No. (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mediastinal malignancy</td>
<td>47 (70.1)</td>
</tr>
<tr>
<td>Lung cancer</td>
<td></td>
</tr>
<tr>
<td>Non-small cell carcinoma, adenocarcinoma</td>
<td>12 (17.9)</td>
</tr>
<tr>
<td>Non-small cell carcinoma, epidermoid epithelioma</td>
<td>18 (26.9)</td>
</tr>
<tr>
<td>Non-small cell carcinoma, large cell</td>
<td>12 (17.9)</td>
</tr>
<tr>
<td>Small cell carcinoma</td>
<td>3 (4.5)</td>
</tr>
<tr>
<td>Other cancers</td>
<td>2 (3.0)</td>
</tr>
<tr>
<td>No mediastinal malignancy</td>
<td>18 (26.9)</td>
</tr>
<tr>
<td>Benign or reactive lymph node tissue</td>
<td>13 (19.4)</td>
</tr>
<tr>
<td>Granulomatous lung disease/sarcoidosis</td>
<td>5 (7.5)</td>
</tr>
</tbody>
</table>

*In two cases (3.0%), no final diagnosis was obtained due to patient-related reasons.

Table 3—Comparison of Test Characteristics of FDG-PET, EUS Without FNA, and EUS-FNA With Mediastinal Malignancy as an End Point

<table>
<thead>
<tr>
<th>Variables</th>
<th>FDG-PET (n = 24)</th>
<th>EUS (n = 65)</th>
<th>EUS-FNA (n = 65)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Malignant ML or LAG prevalence</td>
<td>71.4</td>
<td>72.3</td>
<td>72.3</td>
</tr>
<tr>
<td>Sensitivity</td>
<td>85.0 (62–91)</td>
<td>93.6 (80–96)</td>
<td>100 (92–100)</td>
</tr>
<tr>
<td>Specificity</td>
<td>50.0 (15–84)</td>
<td>27.8 (9–53)</td>
<td>100 (81–100)</td>
</tr>
<tr>
<td>Positive predictive value</td>
<td>81.0 (58–89)</td>
<td>77.2 (64–82)</td>
<td>100 (92–100)</td>
</tr>
<tr>
<td>Negative predictive value</td>
<td>57.1 (18–90)</td>
<td>62.5 (24–91)</td>
<td>100 (81–100)</td>
</tr>
<tr>
<td>Accuracy</td>
<td>75.0</td>
<td>75.4</td>
<td>100</td>
</tr>
</tbody>
</table>

*Data are presented as % or % (95% confidence interval).
In this 1-year series of consecutive patients, EUS-FNA with cytopathologist-assisted ROSE resulted in a high diagnostic overall accuracy without complications and with a high avoidance rate of surgical procedures. The evaluation of lymph nodes with imaging techniques is unsatisfactory. Tissue diagnosis of enlarged ML on CT or mediastinal FDG-PET hot spots is hence still warranted. Retrospective and uncontrolled prospective series reported on EUS-FNA as a tool in lung cancer staging. Its test characteristics not only depend on the procedure characteristics but are also influenced by the selection bias of the study population. In patients with suspect lymph nodes on CT scan, EUS with or without FNA showed a sensitivity of 0.72 to 0.97 and a specificity of 0.81 to 1.00. As these studies originated in gastroenterology departments, the patients were often investigated first with radial EUS (without the possibility of FNA) followed by linear EUS-FNA. By consequence, sensitivity in these series includes both cases of positive FNA findings of ML and of T4 extension shown by means of radial EUS. In patients with mediastinal hot spots on FDG-PET, Kramer et al showed prospectively that EUS-FNA detected 50 of 69 patients with malignant ML, resulting in a sensitivity of 72% for detecting mediastinal malignancy. Similarily, Annema et al showed that EUS-FNA was able to diagnose N2 or N3 in 89%, provided the patient had FDG-PET–positive lesions in the mediastinum. The high accuracy and sensitivity of our series, in patients with suspect ML or LAG, is superior to earlier reports of EUS-FNA. We argue that the presence of a cytopathologist at the ROSE contributed to this result. False-negative rates vary in the literature up to 20% and even 40%. Most centers perform ROSE by a laboratory technician only. Despite ROSE, inconclusive smears represent up to one third of the patients after FNA. ROSE resulted in our series in the absence inconclusive smear results, as we kept puncturing until the cytopathologist indicated there was enough material for making a conclusive pathology report. This approach is confirmed by a study in which ROSE with a cytopathologist significantly improved the yield of endoscopic FNA procedures. Another report also confirmed this finding for TBNA and calculated that a plateau in the yield of malignancy is reached after seven punctures. It is indeed neither practical nor convenient for patients and investigators to perform a standard set of seven to eight punctures in order to avoid ROSE.

Despite the presence of a cytopathologist, we failed to come to a more specific diagnosis in case no malignancy was found with EUS-FNA. The diagnosis of granulomatous ML appears to be especially challenging. Other series reported the possibility of finding granulomas in FNA smears. Wildi et al investigated this in a retrospective series in which 35 of 124 patients had granulomatous ML diagnosed with EUS-FNA, while only 3 patients with sarcoidosis had false-negative findings. However, the main criticism is that the methodology in this study was not uniform since all their patients were investigated with radial EUS followed by linear EUS, and since different-gauged needles (19-gauge) as well as Trucut needles were applied. Moreover, the results were not controlled with an invasive surgical procedure as "gold standard." A recent study performed in consecutive patients with a high suspicion for sarcoidosis indicated a sensitivity of 82% for EUS-FNA. The false-negative samples in this study were explained to be the consequence of ML fibrosis, as often seen in stage II sarcoidosis. The limitations of this observational study are obvious: first, all patients were selected because of abnormal CT or FDG-PET findings at the time of referral. Moreover, an inherent bias is the fact that all patients had ML reachable with EUS. In our series, there were no patients with suspect ML situated uniquely at level 6 (para-aortic ML) or pretracheally. Lastly, we relied for our analysis on a composite "gold standard" that used results obtained by EUS-FNA, surgery, and clinical follow-up. One might reason that all patients should be subjected to a surgical procedure to exclude possible false-positive EUS-FNA results. We judged it unethical to subject patients with malignant disease to further diagnostic surgery in order to verify the obtained results. This is an approach that is comparable to other reports in this field. A clinical relevant consequence remains the fact that a significant number of patients were not referred to the surgeon because of proof of mediastinal involvement after EUS-FNA. This figure of avoided procedures is comparable to that obtained in patients with FDG-PET–positive ML, in which EUS-FNA avoided diagnostic surgery in 62 to 69%. The fact that in our series 40% of the patients were punctured at lymph node regions that are out of reach for cervical mediastinoscopy indicates that an important number of the patients would have undergone thoracotomy or video-assisted thoracoscopy when EUS-FNA was not available. No adverse events were recorded in this study. This compares with findings in other studies performing FNA with 22-gauge needles. Although there is an inherent negative publication bias, there is one report of life-threatening mediastinitis occurring after a cyst was punctured. Another study...
compared the diagnostic yield of a 19-gauge Trucut needles with classic 22-gauge aspiration needles. While Trucut needles did not result in better accuracy, they induced mediastinitis and bleeding. Whether 19-gauge aspiration needles play can play a role in the diagnosis of granulomatous disease remains unclear.

In conclusion, we show that EUS-FNA has an excellent accuracy when performed with cytopathologist-assisted ROSE. We confirm that the technique is feasible, safe, and avoids further surgical staging in a significant number of patients with suspect ML.

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