Modified Technique for Obtaining Mediastinal Samples With Endobronchial Ultrasound-Guided Transbronchial Needle Aspiration: Results From a Prospective Observational Study

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ABSTRACT

Background: Endobronchial ultrasound-guided transbronchial needle aspiration (EBUS-TBNA) is a widely-accepted method for obtaining both benign and malignant mediastinal lymph node samples. We present the results obtained with a modification that simplifies sampling, known as fine-needle capillary sampling or EBUS-FNC.

Methods: A prospective observational study with 44 consecutive patients who underwent EBUS at the University of Navarra Clinic in Pamplona, Spain. All samples were obtained by EBUS-FNC instead of conventional EBUS-TBNA. No suction was used, and the internal stylus was not completely withdrawn at any time.

Results: The examination of the mediastinum by means of EBUS identified the presence of lymphadenopathies or mediastinal masses in 38 patients (86.4%). Samples were taken from more than one lymph node in 23 patients (52.3%). EBUS-FNC provided adequate and representative material for interpretation in all patients, and diagnostic performance was 87%. Sensitivity for the detection of lung cancer with EBUS-FNC was 84%. Mild complications were only recorded in two patients (4.5%).

Conclusions: Our study suggests that EBUS-FNC is a safe technique, comparable to EBUS-TBNA in efficacy, and is able to obtain adequate samples.

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Técnica modificada de obtención de muestras del mediastino mediante punción transbronquial bajo guía de ecografía endobronquial: resultados de un estudio prospectivo observacional

RESUMEN

Antecedentes: La aspiración mediante punción transbronquial bajo guía de ecografía transbronquial (EBUS-TBNA) es un método ampliamente aceptado para la obtención de muestras de ganglios linfáticos mediastínicos tanto benignos como malignos. Se presentan los resultados obtenidos con una modificación que simplifica la técnica de obtención de muestras a la que se denomina capilaridad con aguja fina o EBUS-FNC.

Métodos: Estudio prospectivo observacional de 44 pacientes consecutivos a los que se practicó una EBUS en la Clínica de la Universidad de Navarra de Pamplona, España. Todas las muestras se obtuvieron mediante EBUS-FNC en vez de con la EBUS-TBNA convencional. No se aplicó aspiración y no se retiró en ningún momento por completo el estilete interno.

Resultados: La exploración del mediastino mediante EBUS identificó la presencia de adenopatías o masas mediastínicas en 38 pacientes (86.4%). Se obtuvieron muestras de más de un ganglio linfático en 23 pacientes (52.3%). La EBUS-FNC proporcionó un material adecuado y representativo para realizar la interpretación en todos los pacientes y el rendimiento diagnóstico fue del 87%. La sensibilidad para la detección del cáncer de pulmón con la EBUS-FNC fue del 84%. Se registraron complicaciones menores (6%) tan solo en dos pacientes (4.5%).

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Palabras clave: Broncoscopia, Ecografía endobronquial, Biopsias por punción, Estadaje mediastínico, Cáncer de pulmón

Introduction

Endobronchial ultrasound-guided transbronchial needle aspiration (EBUS-TBNA) has become widely accepted as one of the various methods currently in existence for obtaining mediastinal samples in a range of pathological states.1–7 It is regarded as a minimally invasive technique and is not associated with serious complication.3,5,7 EBUS-TBNA is particularly useful in the mediastinal staging of non-microcytic lung-cancer (NMLC), for which its sensitivity has been estimated at between 89% and 98%, and its specificity 100%, with a negative predictive value (NPV) in excess of 92%.2,8–13 The EBUS-TBNA has proven useful in diagnosing malignancy in lymph nodes, with a sensitivity of 91% and a NPV of 93%.14 Benign tumours, especially sarcoidosis, can also be easily diagnosed by means of EBUS-TBNA.15–17

The success of EBUS-TBNA depends on the adequate training of the endoscopy team, the availability of an experienced cytopathologist to carry out a rapid evaluation (ROSE), and the use of an appropriate technique during the procedure.18–20 The conventional sampling technique for EBUS-TBNA is based on the application of negative pressure or aspiration with a syringe.20 It has been suggested that continuous aspiration optimises the amount of tissue obtained for diagnostic purposes, and keeps the needle in the target lesion.21,22 All the studies presented so far are based on EBUS-TBNA as the preferable procedure for obtaining samples.

Biffod was the first researcher to describe an alternative sampling technique, with the use of a needle and without the application of external pressure, for breast lesions.23 This simplified sampling technique is based on the physical properties of the liquids, which allows a smooth extraction of the material with no need for aspiration. The technique, which is known as fine needle capillary sampling or FNC has also been tried on other organs24–26 and on lymph nodes. FNC can provide cytology samples of a higher quality, and it may be associated with a lesser degree of contamination of the sample due to haemorrhage than is the case with TBNA. In different studies, it has been described as the technique of choice for sampling in endoscopic ultrasound-guided (EBUS). EBUS-FNC is a simplified technique which obviates the need to withdraw the stylet or to aspirate with a syringe. The aims of the present study are to describe this technique and determine its performance capacity in a random group of patients on whom EBUS mediastinal examination is carried out.

Material and Methods

The study included a total of 44 consecutive patients, on whom a EBUS examination of the mediastinum was performed in the Clinic of the University of Navarre in Pamplona, Spain, between 1 January and 31 August 2011. All the patients concerned had been sent for EBUS following the results obtained in image diagnoses in which mediastinal adenopathies were identified or because they were eligible for mediastinal staging, due to the presence of lung cancer. A medical evaluation was carried out by an endoscopist on all the participants in the scheme, with the aim of recording the relevant demographic data, confirming the advisability of such an intervention, obtaining the informed consent of the patients, and excluding any other possible contraindication. In the same way, the researchers reviewed the existing diagnostic images (CT and or PET-TC) before the intervention, with the aim of identifying the presence of mediastinal lesions that could be aspirated, and planning the procedures to be applied.

Technique for Obtaining Samples by Endobronchial Ultrasound-Guided Transbronchial Aspiration

All the EBUSs were carried out in the endoscopy room with the patient in supine decubitus position, under conscious sedation, by means of a combination of midazolam and fentanyl, and topic anaesthesia (lidocaine 2%). The vital constants and arterial oxygen saturation were monitored continuously. A special bronchoscope was used, with an external diameter of 6.9 mm and a work channel of 2 mm (BF-UC260F-OLB; Olympus Ltd., Tokyo, Japan), inserted orally and passed though the orofarynx and vocal chords until it reached the main carina. The probe (frequency MHz) was connected to another image processor (U-E-C60; Olympus Ltd., Tokyo, Japan). The EBUS was carried out with the balloon partially inflated with a saline solution for obtaining a B mode image, and the mediastinal lymph nodes were examined systematically, following the lymph node map proposed by Mountain.22,23 Once a target lymph node or mediastinal mass was clearly identified by means of the EBUS, the colour Doppler ultrasound was activated in order that the best possible location for the aspiration could be identified, and large blood vessels avoided. The diameter of the lymph node was measured at the short axis and its morphological features recorded.

Aspirations were carried out on all the nodes larger than 5 mm that could be visualised, using a special calibre 22 needle (NA-201SX–4022; Olympus Ltd., Tokyo, Japan), making sure that the first aspirations were carried on N3 nodes, if there were any, followed by 2 and then N1, until a diagnosis was achieved, or until a large number of lymphocytes could be observed in at least one sample from each node.

EBUS-FNC was carried out after the point of the needle had been inserted into the interior of the lesion or the lymph node. A stylet was used to clean the residue, cartilage, bronchial epithelium or blood clots out of the needle channels in the usual manner. Subsequently, instead of removing the stylet altogether and performing aspiration with a Vaclo® syringe in the usual way (TBNA), the stylet was partially and continuously withdrawn while the lymph node or mediastinal mass was being probed with the needle with a gentle penetration movement, under direct ultrasonic visualisation. Aspiration was not performed and nor was the internal stylet ever completely removed. Subsequently, the material was collected by means of a capillary action rather than aspiration (FNC). The needle was then removed from the bronchoscope’s work channel, and the stylet was pressed in order to remove the material contained in its interior and place it on a glass microscope slide for immediate staining. The stylet was then completely removed and a syringe was connected to the needle in order to remove the remaining material, which was then placed on a slide, or so that the cellular material could be placed in a test tube with ethanol. Finally, the stylet was once again inserted into the needle and, if necessary, a new sample was obtained in exactly the same way. All the procedures were carried out by means of ROSE.

ROSE

For each aspiration, at least one glass slide was examined by means of fast-stain, using the Diff-Quik method, by a suitably skilled cytology technician. The preparation was immediately examined.
under a microscope and classified as diagnostic and/or adequate. The remaining preparations were fixed in ethanol (96%) for later examination. The criteria for classifying a sample as adequate were established by Davenport. A sample was considered adequate when the presence of malignant cells or, alternatively, of a benign diagnosis, was evident to the cytopathologist, or when the lymphocytes constituted at least 30% of the total number of cells. The acellular samples or those that showed an abundance of red blood corpuscles or respiratory epithelium cells, were considered inadequate. The number of needle passes required depended on the examination carried out by the cytopathologist of the available material; as well particular care was being taken to ensure that an amply sufficient number of cells could be obtained for analysis of genetic mutation, including analyses of ALK, EGFR and KRAS, which are carried out systematically in our centre when NMLC is confirmed.

### Statistical Analysis

All the variables were recorded and analysed using the data processing programme SPSS 15.0 for Windows, version 15.0.1, November 2006, created by LEAD Technologies Inc. The means are presented together with standard deviation, and a confidence interval of 95%. The frequencies are expressed as percentages.

### Results

The study included 44 patients (75% male) with an average age of 62.2 (±9.8) years. The characteristics of the patient are shown in **Table 1**.

<table>
<thead>
<tr>
<th>Tobacco-related antecedents</th>
<th>79.5% (35)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Packets-years, mean</td>
<td>51.8±28.4</td>
</tr>
<tr>
<td>Malignant illness antecedents</td>
<td>33.3% (14)</td>
</tr>
<tr>
<td>Kidney</td>
<td>4</td>
</tr>
<tr>
<td>Urinary bladder</td>
<td>3</td>
</tr>
<tr>
<td>Lung</td>
<td>2</td>
</tr>
<tr>
<td>Prostate</td>
<td>1</td>
</tr>
<tr>
<td>Breast</td>
<td>1</td>
</tr>
<tr>
<td>Others</td>
<td>3</td>
</tr>
</tbody>
</table>

Examination recommended

<table>
<thead>
<tr>
<th>Examination recommended</th>
<th>Suspect of malignant disease</th>
<th>82% (36)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Examination of panenchymal/mediastinal anomalies</td>
<td>18% (8)</td>
</tr>
</tbody>
</table>

Obvious primary tumour

| Upper right lobe          | 90.9% (40) |
| Central lobe              | 5          |
| Lower right lobe          | 5          |
| Upper left lobe           | 6          |
| Lower left lobe           | 5          |
| Hilar                     | 5          |
| Mediastinum               | 2          |
| Other location            | 7          |

Puncture of lymph gland 86.4% (38)

| Sampling stations         | 4R | 18.5% |
|                          | 4L | 6.2%  |
|                          | 5  | 15.4% |
|                          | 7  | 29.2% |
|                          | 10R| 10.8% |
|                          | 10L| 9.2%  |

Endobronchial lesion 10.7%

Number of puncture sites

| No puncture               | 4.5% (2) |
| Only one site             | 43.2% (19) |
| 2 or more sites           | 52.3% (23) |

Total number of FNC applications 192

Average number of applications 5

Complications associated with the technique 4.5% (2)

The final diagnosis was lung cancer in 61.4% (27/44) of the patients, with a greater incidence in men (81.5%), and in patients with tobacco-related antecedents (89.9%). Other diagnoses were metastasis in the lymph nodes, renal, skin and breast neoplasms, nontuberculosis mediastinal granulomatous lymphadenitis (2 cases), plasmocytoid cell tumour (1 case) and reactive lymphadenopathy with an obvious focus of infection (2 cases).

In 4 of the 7 cases in which EBUS-FNC did not permit a definitive diagnosis, the procedure applied to establish diagnosis was correlation with clinical antecedents, diagnostic image examination
and reports from the Cytology Department in relation to the samples.

Discussion

The diagnostic performance of the EBUS-TBNA depends on a number of factors, including the following: the availability of recent diagnostic images for an adequate planning of the intervention, adequate experience on the part of the staff involved, the availability of ROSE, obtaining an adequate representative sample from the lymph gland.\textsuperscript{18-20,35-38} The conventional technique for obtaining samples using EBUS or TBNA requires the application of negative pressure.\textsuperscript{20} The sample is obtained primarily by means of aspiration. It has been said that this method reduces the presence of inadequate samples by 5\% or more.\textsuperscript{22-38} Concerns about the influence of FNA on the quality of the sample were first voiced by Zajdela et al.\textsuperscript{24} This author described contamination of the sample resulting from bleeding attribute to the wounding of the neighbouring tissue, which complicated the cytopathological evaluation of mammary samples obtained by means of FNA. FNC was recommended as an alternative means of obtaining samples. This method was introduced by Bradford in 1982 in a study of mammary carcinoma samples. This key study highlighted the fact that there is a good correlation between cytological samples obtained by means of FNC and histology.\textsuperscript{23}

FNC is based on capillarity, a physical property of liquid and semi-fluid media which permits the surface of a liquid to flow spontaneously towards the interior of a capillary tube. This effect is the result of the cohesion forces, known as surface tension, which are present between the molecules of the liquid. The analysis of the liquid is directly proportional to the surface tension, and inversely proportional to the calibre of the tube.\textsuperscript{39} FNC can be useful as a means of obtaining lymph node samples, as the cells are not bonded in a compact ultrastructure, which means that material can rise through the interior of the needle by capillarity as the stylet is withdrawn.\textsuperscript{25} FNC can provide cellular material and its diagnostic usefulness is comparable to that of conventional FNA, according to data obtained in patients with breast cancer in the Curie Institute in France.\textsuperscript{24} In comparative studies carried out on thyroid glands and the liver, a distortion of the cellular architecture of the samples obtained by conventional FNA has been described,\textsuperscript{25,26} although no significant differences were observed in the diagnostic usefulness of FNC as opposed to FNA (sensitivity of 90\% compared with 80\%-90\%),\textsuperscript{25,40} FNC can be considered superior to FNA as contamination resulting from blood is minimal, and the cellular morphology is better preserved.\textsuperscript{41,42} This serves to explain the greater diagnostic usefulness compared with FNA.\textsuperscript{24,26,41-43}

The question of whether FNC is more advantageous than conventional FNA in EUS remains to be answered. Wallace and Storch did not observe any significant differences in terms of the quality or diagnostic usefulness of the samples obtained by means of EUS-FNA compared with FNC.\textsuperscript{28,29} In Wallace’s study, FNA was more likely to produce sanguineous samples, but it also produced more cellular samples than FNC.\textsuperscript{28} The authors arrived at the conclusion that FNC results in a greater overall sample quality as less blood is present, although there were no differences in terms of the diagnostic usefulness. Puri et al., by contrast, described a greater sensitivity and negative predictive value in samples obtained by means of EUS-FNA than in those obtained by EUS-FNC (85.7\% as opposed to 66.7\%).\textsuperscript{30}

One of the advantages of EUS-FNC compared with EUS-FNA could be the need for fewer needle passes. It has been pointed out that the optimal number of needle passes with conventional FNA is 5 for lymph glands and 7 for solid tumours, with an overall sensitivity of 77\% and 83\%, respectively, and a specificity of 100\%.\textsuperscript{44,45} Wallace, by contrast, suggests that 3 is the optimal necessary number for a diagnosis based on samples obtained by means of EUS-FNC.\textsuperscript{28} Given that each needle pass takes between 3 and 5 min from beginning to end, fewer passes would substantially reduce the length of time required for the intervention and therefore the length of the sedation required, which could result in a smaller number of possible EUS complications.

Our study supports the use of FNC as a viable alternative to TBNA in the samples obtained by means of EBUS. Comparisons with historical data published so far suggest that the techniques are, to say the least, comparable, as regards their effectiveness in obtaining adequate samples and as regards diagnostic accuracy, given that 77\%-100\% of the samples obtained with EBUS-TBNA form, are adequate and/or diagnostic.\textsuperscript{13,27,46} It is possible to argue that FNC is easier to use it avoids the need for needle aspiration, which simplifies the process of obtaining lymph gland samples in the mediastinum. There is a consensus among practitioners with experience in EBUS and conventional TBNA that EBUS-TBNA is a laborious technique which often requires at least two specialists in order to obtain a single sample, although there are some bronchoscopists who are used to applying the aspiration while they hold the needle and the bronchoscope with one hand. Simplifying the technique could be useful in EBUS examination of the mediastinum in an overburdened working environment in which the endoscopist has to deal with sedation, bronchoscopy, monitoring of the patient and the obtaining of tissue all at the same time.

To the best of our knowledge, this is the first study which systematically focuses on the obtaining of mediastinal samples with EBUS using FNC as opposed to conventional TBNA. One of the limitations of our study is the lack of comparisons between EBUS-FNC with conventional EBUS-TBNA. In this respect it should be seen as a hypothesis-generating study and proof of concept. Another serious limitation is the prevailing uncertainty regarding what constitutes an adequate sample. It should be pointed out that there are no established criteria in this regard. Consequently, the evaluation of a given sample as adequate or inadequate can be considered subjective; in that it depends on the criteria of the specialist, and may not be reproducible. By and large, little coincidence among observers appears to be the order of the day.\textsuperscript{31}

Conclusions

Our study indicates that EBUS-FNC is a viable alternative to conventional EBUS-TBNA for obtaining mediastinal samples. The modified technique is safe and comparable, in terms of its effectiveness and its success in obtaining adequate samples, to everything that has been published in relation to the performance of EBUS-TBNA. It is arguable that EBUS-FNC is simpler than EBUS-TBNA and can be better in the sense that it can reduce the amount of time required for obtaining samples, and in terms of the complexity of the procedure. Whether EBUS-FNC provides better quality samples remains to be demonstrated by means of a prospective and randomised study.

Conflict of Interest

The authors have no conflict of interest to declare.

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