REFINEMENTS AND INNOVATIONS IN BIOPSY AND ANALYSIS TECHNIQUES FOR PLEURAL AND LUNG DISEASE

ANDREAS HENRI DIACON

DISSERTATION PRESENTED FOR THE DEGREE OF DOCTOR OF PHILOSOPHY
AT THE UNIVERSITY OF STELLENBOSCH

PROMOTOR:

PROF CHRIS T BOLLIGER

CO-PROMOTORS:

PROF COLLEEN A WRIGHT

PROF GERHARD WALZL

DECEMBER 2007

Declaration

I, the undersigned, hereby declare that the work contained in this dissertation is my own original work and that I have not previously in its entirety or in part submitted it at any university for a degree.

Signature: Date:

1. Abstract

1.1. Background

Tumors arising from the lung, pleura, or chest wall are a frequent problem in clinical pulmonary medicine. Most lesions are either infectious, neoplastic or granulomatous in nature, but a variety of other differential diagnoses must be considered. An accurate diagnosis is important because the available treatments differ substantially, and because any delay will impair the prognosis in potentially curable patients with lung carcinoma. The investigations involve the disciplines of radiology, pulmonology, surgery, microbiology, and anatomical pathology and consume a respectable amount of resources. The aim of the work covered in this thesis was to optimize the available diagnostic methods for the routine use in a health care setting with limited resources.

1.2. Methods

The general idea of this work was to identify conventional sampling methods that could be developed further to become more useful for the diagnosis of chest tumors in a low resource health care setting. The key method was research performed: a) to revise and expand the indication for a sampling method, b) to technically improve the sampling process, and c) to optimize sample transport, preparation and analysis in collaboration with the analytical laboratory.

1.3. Results

A list of invasive diagnostic procedures, imaging methods and analytical processes were developed, evaluated and integrated into clinical practice. A) transbronchial needle aspiration, B) transthoracic cutting needle biopsy, C) transthoracic fine needle aspiration, D) transthoracic ultrasound, and E) rapid on-site evaluation of needle aspirates by a cytopathologist. Five studies pertaining to this thesis were published in international peer-reviewed journals:

- ***Safety and yield of ultrasound-assisted transthoracic biopsy performed by pulmonologists (Respiration 2004;71:519-22)***

This paper established that ultrasound-assisted transthoracic biopsy performed by pulmonologists is feasible, safe, practical, low-cost and has a high yield.

- ***Utility of rapid on-site evaluation of transbronchial needle aspirates (Respiration 2005;72:182-8)***

This paper demonstrated the economical advantages of on-site evaluation of transbronchial specimens in a low-resource setting.

- ***Transbronchial needle aspirates: comparison of two preparation methods (Chest 2005;127:2015-8)***

This paper demonstrated that preparing smears on-site has a far better yield than pooling samples into a vial. This means that the yield is improved over the current standard at no additional cost.

- ***Transbronchial needle aspirates: how many passes per target site? (European Respiratory Journal 2007;29:112-6)***

This paper investigated the most economical and effective approach to serial sampling with transbronchial needle aspiration during flexible bronchoscopy.

- ***Ultrasound assisted transthoracic biopsy: fine needle aspiration or cutting needle biopsy? (European Respiratory Journal 2007;29:357-62)***

This paper compared two common methods of sampling and demonstrates that the less expensive method is sufficient in the majority of cases.

1.4. Conclusion

This work has impacted on current practice in multiple ways. Conventional methods have been optimized by improving technical factors and with the integration of interdisciplinary collaboration. The initiated research is ongoing with the aim to achieve continued technical and economical improvements in the diagnosis of chest tumors.

2. Abstrak

2.1. Agtergrond

Tumore van die long, pleura of borskaswand word gereeld geëvalueer in kliniese pulmonolgie. Meeste letsels is infektief, neoplasties of granulomateus, maar heelwat ander patologië moet oorweeg word in die differensiële diagnose. 'n Akkurate en spoedige diagnose is belangrik, nie aanleenlik omdat die beskikbare hantering beduidend verskil nie, maar ook omdat 'n vertraging die prognose van potensieël geneesbare long tumore kan verswak. Spesiale ondersoeke betrek die dissiplines van Radiologie, Pulmonologie, Chirurgie, Mikrobiologie en Anatomiese Patologie en is oor die algemeen duur. Die doel van die navorsing wat die basis vorm van hierdie tesis was om die beskikbare diagnostiese tegnieke te optimaliseer en aan te pas vir roetine gebruik in 'n gesondheidsisteem met beperkte finansiële middele.

2.2. Metodes

Konvensionele metodes wat ingespan word tydens die verkryging van laboratorium monsters is geïdentifiseer en geoptimaliseer met die doel om hierdie tegnieke meer bruikbaar te maak in die diagnose van tumore van die toraks in 'n gesondheidsisteem met beperkte fondse. Navorsing is gedoen: a) om die indikasies vir bepaalde metodes tydens die neem van monsters te hersien en uit te brei, b) om die proses van monsterneming te optimaliseer, en c) om die vervoer, voorbereiding en analise van monsters in samewerking met die betrokke laboratoria te optimaliseer.

2.3. Uitslae

'n Lys van invasiewe diagnostiese prosedures, beeldings tegnieke en analitiese prosesse is ontwikkel en geïntegreer in kliniese gebuik. A) transbrongiale naald aspirasie, B) transtorakale snynaald biopsie, C) transtorakale fynnaald aspirasie, D) transtorakale ultraklank, and E) spoedige in-teater evaluasie van naald aspirate deur sitopatoloë. Vyf studies wat betrekking het tot hierdie tesis is gepubliseer in internasionale joernale:

- ***Safety and yield of ultrasound-assisted transthoracic biopsy performed by***

pulmonologists (Respiration 2004;71:519-22)

Hierdie studie het bevestig dat ultraklank-geassisteerde transtorakale biopsies wat deur 'n pulmonoloog uitgevoer is, haalbaar, veilig, prakties en goedkoop is en dat dit 'n hoë opbrengs het met betrekking tot diagnose.

- ***Utility of rapid on-site evaluation of transbronchial needle aspirates (Respiration 2005;72:182-8)***

Hierdie publikasie het die ekonomiese voordele van spoedige in-teater evaluasie van transbrongiale monsters bevestig in 'n gesondheidsisteem met beperkte fondse.

- ***Transbronchial needle aspirates: comparison of two preparation methods (Chest 2005;127:2015-8)***

Hierdie studie het bewys dat in-teater voorbereiding van skyfies ‘n baie beter diagnostiese opbrengs lewer as om al die aspirate in ‘n enkele houer in te spuit. Dit het impliseer dat die opbrengs verbeter is sonder enige addisionele kostes.

- ***Transbronchial needle aspirates: how many passes per target site? (European Respiratory Journal 2007;29:112-6)***

Hierdie studie het die mees ekonomiese en effektiewe benaderings tot die seriële monsterneming met transbrongiale naald aspirasie gedurende veselskope ondersoek.

- ***Ultrasound assisted transthoracic biopsy: fine needle aspiration or cutting needle biopsy? (European Respiratory Journal 2007;29:357-62)***

Hierdie artikel het twee algemene metodes van monsterneming vergelyk en het getoon dat die goedkoper metode voldoende is in die meerderheid van gevalle.

2.4. Gevolgtrekking

Hierdie navorsing het ‘n beduidende inpak gehad op huidige kliniese praktyk. Konvensionele metodes is geoptimaliseer deur tegniese faktore te verbeter en deur die integrasie van interdissiplinêre samewerking. Die geïnisieërde navorsing is voortgaande en streef daarna om nuwe tegniese en ekonomiese verbeteringe in die diagnose van tumore van die toraks te bewerkstellig.

3. Table of Contents and Abbreviations

Abbreviations

CT: Computed tomography

ROSE: Rapid on-site evaluation

TBNA: Transbronchial needle aspiration

TTNA: Transthoracic needle aspiration

US: Ultrasound

4. Background, literature review and research plan

4.1. Chest tumors

Tumors of the the chest represent a common clinical problem for the chest physician. For the purpose of this work, chest tumors as a group are functionally defined as abnormal chest masses detectable on a radiograph, which can arise from the lungs, the pleura, the anterior mediastinum and the chest wall. Examination of chest tumors is an interdisciplinary task which requires close collaboration of pathologist, physician and radiologist.

4.1.1. Lung tumors

Lung tumors are best classified into benign (infectious granuloma, benign neoplasms, inflammatory, other) and malignant (small cell and non small cell bronchogenic carcinoma, carcinoid, metastatic lesions). The most frequent lung mass is primary lung cancer, which is currently the most common cause of cancer mortality throughout the world [1]. The major histologic types of lung cancer include small cell carcinoma and the non-small cell carcinoma types of squamous cell carcinoma, adenocarcinoma, and large cell carcinoma [2]. The International Staging System for non-small cell lung cancer relies on scoring the extent of the primary tumor (T), lymph node involvement (N) and the presence of distant metastases (M), the combination of which will define a clinical stage I-IV [3]. The single most important prognostic factor is mediastinal lymph node involvement.

4.1.2. Pleural tumors, anterior mediastinal tumors, chest wall tumors

Malignant pleural mesothelioma is by far the most frequent pleural tumor. Its differential diagnosis is not wide but includes both benign and malignant processes [4]. The main challenge in diagnosing malignant mesothelioma is the distinction from metastatic adenocarcinoma. Possible primary sources for the latter include lung, breast, stomach, kidney, ovary, and prostate.

Lesions most commonly found in the anterior mediastinum are thymomas, germ cell tumors, lymphomas, intrathoracic thyroid tissue, and parathyroid lesions. Thymomas are the most common anterior mediastinal primary neoplasm in adults [5]. Primary mediastinal lymphoma

accounts for 10% to 20% of primary mediastinal masses and is the second most common primary anterior mediastinal mass [6]. Germ cell tumors account for 15% of anterior mediastinal tumors in adults [7].

Most tumors clinically imposing as derived from the chest wall are in fact metastases from distant primary tumors or tumors extending from the lung or pleura into the chest wall. The differential diagnosis of genuine chest wall tumors includes benign or malignant neoplasms of bone and cartilage (osteochondroma, osteochondrosarcoma). Rarely, a solitary plasmacytoma can present as a lytic rib lesion.

4.2. Sampling techniques

In pursuing a tissue diagnosis of a chest tumor, there is a range of procedures to choose from. If there is evidence of supraclavicular nodal involvement or of an easily accessible metastatic site such as a pleural effusion, then these should be assessed first. Sputum cytology is a non-invasive study with a high specificity, but a positive finding of cancer is only helpful if the patient is not a surgical candidate because of anatomic location of the lesion or severe physiologic limitations. All other patients will undergo either bronchoscopy or a transthoracic biopsy.

4.2.1. Transbronchial needle aspiration (TBNA)

TBNA is an elegant and effective sampling method [8]. A short, protected and disposable needle attached to a flexible plastic catheter is introduced via flexible bronchoscope and can be inserted into the lesion of interest under direct vision [9]. In lung cancer, TBNA often establishes the diagnosis and provides staging information in a single procedure [10]. Although of proven value also in non-malignant and in infectious disease TBNA remains underutilized [11, 12]. One reason might be that there are still aspects of TBNA that await clarification. Technical issues are for instance the number of needle passes that are required to provide a diagnosis at a certain site, and whether this number of passes varies in different clinical situations.

4.2.2. Transthoracic needle aspiration (TTNA) and ultrasound (US)

TTNA of the lung is a well-established method in the diagnosis of pulmonary nodules. The reported diagnostic accuracy of computed tomography (CT) guided TTNA is greater than 80% for benign disease and greater than 90% for malignant disease [13]. Transthoracic ultrasound (US) of the chest is useful in the evaluation of a wide range of peripheral parenchymal, pleural, and chest wall diseases [14]. A recent review about ultrasound techniques for chest physicians published by the applicant is attached [15]. Although US is inexpensive, comfortable, mobile and relatively easy to learn, most chest physicians are not familiar with US. In order to promote US guided biopsy as a routine tool for chest physicians a straight forward, simple and safe method to perform US guided biopsies needs to be developed. However, it is unclear whether the number of patients with suitable tumors is large enough for sustaining such a routine procedure. Also, it is not clear if chest physicians can reach the same levels of safety and yield as specialist radiologists.

4.2.3. Sample types and sample handling

The importance of proper handling of the obtained specimen is a critical aspect of biopsy procedures which is in stark contrast to the paucity of literature covering the subject. Work needs to be done to establish standard protocols as to how to harvest good specimens from chest tumors for cytology. In TTNA, it is unclear whether a histological sample or a fine needle aspiration are preferable for safety and yield. In TBNA, it is unclear whether needle aspirates should be smeared directly or conserved in alcohol for later processing [16].

One of the most important issues in tumor diagnosis is close communication between pathologist and clinician. Rapid on-site evaluation (ROSE) of the specimen by a cytopathologist present in theatre has been demonstrated to increase the diagnostic yield in transbronchial needle aspiration [17, 18]. However, ROSE is not widely practiced and has never been validated for transthoracic samples.

4.3. Integration of sampling and analysis

Given the economical pressure the local health care system is being subjected to, it is clear that diagnostic procedures in chest tumors must be developed to preferably employ the safest,

least invasive, and least costly tests in a given situation. For the potentially operable patient with suspected lung cancer, bronchoscopy with direct examination of the visible airways is the preferred initial invasive diagnostic procedure. The principal goal is to diagnose and pathologically stage the patient's tumor at the same time. This can be achieved with TBNA applied to the primary lesion and mediastinal nodes. There is scope for technical refinements as well as for collaboration with cytopathologists for the analysis of samples.

Patients with potentially resectable lesions after non-contributory bronchoscopy, patients with suspected non-resectable lung cancer or patients who prefer conservative treatment over surgery are candidates for a transthoracic procedure. For larger peripheral-based lung tumors, pleural, chest wall and anterior mediastinal lesions, ultrasound is an ideal but underused guiding method of choice for the physician. A practical method needs to be developed for US-assisted fine needle aspiration and for core biopsy, and the respective indications for both need to be clarified. Further, ROSE confirming adequacy of the retrieved specimen could avoiding the need for a histological biopsy.

5. Outline

5.1. Aim

To better integrate transbronchial needle aspiration, transthoracic ultrasound guided needle biopsy as well as rapid on-site analysis of samples in theatre into clinical routine practice.

5.2. Objectives

- To explore TBNA as the leading bronchoscopic sampling tool
- To develop an ultrasound-guided TTNA technique
- To improve sample collection and processing techniques for TBNA and TTNA
- To integrate cytopathological ROSE for TBNA and TTNA
- To investigate synergisms between ROSE and needle aspiration techniques
- To estimate the economical impact of these methods

5.3. Methods and logistics

This work was subdivided into single goal-orientated studies which are designed to result in independent scientific publications. These publications are the output of this work.

5.4. Statistical aspects

Expert statistical support has been received for each study if required (Martin Kidd, Center for Statistical Consultation, University of Stellenbosch). The statistical aspects are study specific and described in detail in the single papers.

5.5. Ethics

Most of this work consisted in observation, documentation and analysis of routine clinical procedures. Ethics Committee approval was obtained for studies with TBNA and on-site cytology (project number: 2003/180) and for transthoracic biopsy (project number: 2003/177).

5.6. Finances and locations

The applicant was supported by a post-doc research grant by the University of Stellenbosch.

6. Published studies derived from this thesis

Five studies published by the applicant as the first author in international peer-reviewed journals form the main part of this thesis. The abstracts of the studies are displayed on the following pages. The complete studies are attached as Appendix 1-5.

- ***Safety and yield of ultrasound-assisted transthoracic biopsy performed by pulmonologists [19]***

  Respiration 2004;71:519-22

- ***Utility of rapid on-site evaluation of transbronchial needle aspirates [20]***

  Respiration 2005;72:182-8

- ***Transbronchial needle aspirates: comparison of two preparation methods [21]***

  Chest 2005;127:2015-8

- ***Transbronchial needle aspirates: how many passes per target site? [22]***

  European Respiratory Journal 2007;29:112-6

- ***Ultrasound assisted transthoracic biopsy: fine needle aspiration or cutting needle biopsy? [23]***

  European Respiratory Journal 2007;29:357-62

6.1. Study 1

6.1.1. Citation:

Diacon AH, Schuurmans MM, Theron J, Schubert PT, Wright CA, Bolliger CT. Safety and yield of ultrasound-assisted transthoracic biopsy performed by pulmonologists. Respiration 2004;71:519-522.

6.1.2. Abstract:

BACKGROUND: Transthoracic ultrasound (US) has gained popularity as a tool for visualizing pleural effusions and assisting thoracentesis or chest drain placement. In the absence of effusion, US just as well demonstrates solid masses involving or abutting the pleura, yet biopsy of such lesions is not widely performed by chest physicians.

OBJECTIVE: To assess the feasibility and the safety of US-assisted cutting needle biopsy performed by chest physicians in routine practice.

METHODS: Lesions involving or abutting the pleura > or =20 mm in diameter on US were sampled with a 14-gauge cutting needle under local anesthesia. Biopsy site, needle direction and depth of penetration were determined with US. The procedure was performed without direct US guidance in 'free-hand' technique.

RESULTS: Ninety-one patients underwent 96 cutting-needle biopsies for suspected peripheral lung tumors (n = 44, 46%), pleural-based (n = 39, 41%), mediastinal (n = 10, 10%), or chest wall lesions (n = 3, 3%), which were single in 71%, multiple in 6% and diffuse in 23%. Sensitivity for malignant neoplasms (n = 65) was 85.5% and 100% for mesothelioma (n = 10). Pneumothorax occurred in 4%.

CONCLUSIONS: US-assisted cutting-needle biopsy of lesions > or =20 mm in diameter is safe in the hands of pulmonologists. The yield for neoplastic disease including mesothelioma is high.

6.2. Study 2

6.2.1. Citation:

Diacon AH, Schuurmans MM, Theron J, Louw M, Wright CA, Brundyn K, Bolliger CT. Utility of rapid on-site evaluation of transbronchial needle aspirates. Respiration 2005;72:182-188.

6.2.2. Abstract:

BACKGROUND: Rapid on-site evaluation has been proposed as a method to improve the yield of transbronchial needle aspiration.

OBJECTIVES: This study investigated whether on-site analysis facilitates routine diagnostic bronchoscopy in terms of sampling, yield and cost.

METHODS: Patients with lesions accessible for transbronchial needle aspiration on computed tomography were investigated. A cytopathologist screened the needle aspirates on site for the presence of diagnostic material. The bronchoscopic sampling process was adjusted according to the results. In 90 consecutive patients with neoplastic disease (n=70; 78%), non-neoplastic disease (n=16; 18%) or undiagnosed lesions (n=4; 4%) we aspirated 162 lung tumors or lymph node sites (mediastinal: 7%; tracheobronchial: 68%; other: 25%).

RESULTS: The diagnostic yield of needle aspiration was 77 and 25% in patients with neoplastic and non-neoplastic lesions, respectively. Sampling could be terminated in 64% of patients after needle aspiration had been performed as the only diagnostic modality, and on-site analysis identified diagnostic material from the first site aspirated in 50% of patients. Only in 2 patients (2%) diagnostic aspirates were not recognized on site. On-site analysis was cost effective due to savings for disposable diagnostic tools, which exceeded the extra expense for the on-site cytology service provided.

CONCLUSIONS: Rapid on-site analysis of transbronchial aspirates is a highly useful, accurate and cost-effective addition to routine diagnostic bronchoscopy.

6.3. Study 3

6.3.1. Citation:

Diacon AH, Schuurmans MM, Theron J, Brundyn K, Louw M, Wright CA, Bolliger CT. Transbronchial needle aspirates: comparison of two preparation methods. Chest 2005;127:2015-8.

6.3.2. Abstract:

STUDY OBJECTIVES: Transbronchial needle aspiration has evolved as a key bronchoscopic sampling method. Specimen handling and preparation are underrated yet crucial aspects of the technique. This study was designed to identify which of two widely practiced sample preparation methods has a higher yield.

DESIGN: Prospective comparison of two diagnostic methods.

SETTING: Tertiary academic hospital.

PATIENTS: Consecutive patients undergoing transbronchial needle aspiration.

INTERVENTIONS: Transbronchial aspirates were obtained pairwise. One specimen was placed directly onto a slide and smears were prepared on site (ie, the direct technique), and the other specimen was deposited into a vial containing 95% alcohol and further prepared in the laboratory (ie, the fluid technique). In total, 282 pairs of samples were aspirated from 145 target sites (paratracheal, 10 sites; tracheobronchial, 101 sites; hilar, 17 sites; endobronchial or peripheral, 17 sites).

MEASUREMENTS AND RESULTS: The measured outcome was the presence of diagnostic material at the final laboratory assessment. At least one diagnostic aspirate was obtained in 66% of 86 investigated patients (small cell lung cancer, 18 patients; non-small cell lung cancer, 47 patients; other diagnoses, 21 patients). The direct technique had a better yield overall than the fluid technique (positive aspirates, 36.2% vs 12.4%, respectively; $p < 0.01$), as well as after stratification for tumor type and for anatomic site.

CONCLUSION: The direct technique is superior to the fluid technique for the preparation of transbronchial needle aspirates.

6.4. Study 4

6.4.1. Citation:

Diacon AH, Schuurmans MM, Theron J, Brundyn K, Louw M, Wright CA, Bolliger CT. Transbronchial needle aspirates: how many passes per target site? European Respiratory Journal 2007;29:112-6.

6.4.2. Abstract:

Transbronchial needle aspiration is a bronchoscopic sampling method for a variety of bronchial and pulmonary lesions. This study investigated whether and how serial needle passes contribute to the yield of transbronchial needle aspiration at specific target sites.

We prospectively recorded 1562 needle passes performed at 374 target sites rated for anatomical location, size, bronchoscopic appearance and underlying disease in 245 patients with neoplastic disease (82%), non-neoplastic disease (15%) or undiagnosed lesions (3%).

Positive aspirates were obtained in 75% of patients and in 68% of target sites. A diagnosis was established with the first, second, third and fourth needle pass at 64%, 87%, 95% and 98% of targets, respectively. The absolute yield varied strongly with target sites features, but the stepwise increment to the maximum yield provided by serial passes was similar across target sites.

Three transbronchial needle passes per site are appropriate when only a tissue diagnosis is sought and when alternative sites or sampling modalities are available. At least four or five passes should be carried out at lymph node stations critical for staging of lung cancer.

6.5. Study 5

6.5.1. Citation:

Diacon AH, Theron J, Schubert P, Brundyn K, Louw M, Wright CA, Bolliger CT. Ultrasound assisted transthoracic biopsy: fine needle aspiration or cutting needle biopsy? European Respiratory Journal 2007;29:357-62.

6.5.2. Abstract:

This study compared the diagnostic yield of ultrasound assisted cutting needle biopsy (CNB) and fine needle aspiration biopsy (FNAB) in chest lesions.

A physician performed ultrasound and FNAB with a 22G spinal needle in all patients directly followed by 14G CNB in patients without contraindication.

We prospectively included 155 consecutive lesions arising from the lung (74%), pleura (12%), mediastinum (11%), or chest wall (3%) in patients with a final diagnosis of lung carcinoma (74%), other malignant tumors (12%), non-neoplastic disease (9%), or unknown (5%). The overall diagnostic yield was 87%. Combined specimens were obtained in 123 lesions (79%). In these, yields of FNAB, CNB and both methods combined were 82%, 76% and 89%, respectively. FNAB was superior to CNB in lung carcinoma (95% versus 81%, p=0.006), but CNB was superior in non-carcinomatous tumors and in benign lesions. On-site cytology was 90% sensitive and 100% specific for predicting a positive FNAB. One patient required drainage for pneumothorax (0.6%).

Ultrasound assisted FNAB performed by chest physicians is an accurate and safe initial diagnostic procedure in patients with a high clinical probability of lung carcinoma. All other patients should undergo concurrent FNAB and CNB.

7. Discussion

7.1. General

This work has shown that improvements in biopsy and analysis techniques in tumors arising from the chest wall, pleura, anterior mediastinum and lung can be achieved by innovative use of existing resources in a setting with limited access to cutting edge technology. The substance of the work is probably best illustrated with the example of TBNA, a field where several research groups around the globe compete in developing strategies to improve TBNA yield and accuracy. It is becoming more and more evident that the regular use of TBNA for staging of lung cancer significantly reduces the number of futile surgical interventions in patients whose disease is too advanced for curative resection [24, 25]. Despite the high relevance of a positive TBNA, the major limitation of TBNA is the widely variable sensitivity that ranges between 39% and 78% in recent meta analyses [26, 27]. Across studies, the prevalence of mediastinal metastasis in the study population is the main determinant of sensitivity. Other factors include the size and location of the lesions, the kind of needle employed, the number of aspirates performed, and the experience and the skill of the operator [28, 29]. Because TBNA for staging of lung cancer is basically a blind procedure, different guiding systems for TBNA have been proposed to improve sensitivity. The first proposed guidance system to direct TBNA was CT-fluoroscopy with a reported increase in TBNA sensitivity by 40% [30]. However, this method is cumbersome and did not gain popularity. An established TBNA guidance system is transbronchial ultrasound (EBUS). In a randomised controlled trial with 200 patients traditional TBNA was compared with EBUS-guided TBNA. The latter makes use of a flexible ultrasound probe introduced through the working channel of the bronchoscope to locate the target lesion. There were no significant differences in sensitivity between conventional TBNA and EBUS guided TBNA for right paratracheal and subcarinal lymph nodes, but significantly better results for left paratracheal and high paratracheal lymphnodes [31]. An even newer type of bronchoscope allows TBNA and endosonography to be performed simultaneously instead of sequentially, so that the tip of the needle can be observed in real time while it is being inserted into the target. Initial results obtained with this method report a sensitivity surpassing 90%, regardless of the lymph node

location or size [32-34]. A further technology recently proposed to guide TBNA is an electromagnetic navigation system, which guides the tip of the bronchoscope to a lesion based on a three dimensional CT reconstruction of the patient's lung and tracheobronchial tree. The reported sensitivity value of 100% will need to be confirmed with further studies [35]. ROSE, the technique developed with the work presented in this thesis, takes a different approach to increasing TBNA yield. While all the technical innovations cited above at best demonstrate in "real-time" that a sample has been harvested from a lesion of interest, ROSE provides "real-time " qualitative information about that sample's diagnostic significance. Compared to the techniques mentioned above, ROSE does not only cost very little but also provides the opportunity to terminate a procedure when its diagnostic objective has been met.

There is considerably less scientific activity on the field of transthoracic ultrasound than with TBNA. US has been overlooked by chest physicians for many years, despite its multiple advantages for the practice of chest medicine. Several review articles have recently emphasized the importance of US for the chest physician. US can be performed by means of the most basic equipment. It can investigate chest wall abnormalities, pleural thickening and pleural tumors as well as describe qualitative and quantitative aspects of a pleural effusion. US can visualise lung tumors and other parenchymal pulmonary processes provided they abut the pleura, and it is the ideal tool to assist with thoracocentesis and drainage of effusions [36-38]. A recent publication confirmed that US is useful for the exclusion of a post-procedural pneumothorax [39].

7.2. Impact of this work on our clinical practice

A very active interdisciplinary collaboration with the Department of Anatomical Pathology has been established and is ongoing on a clinical as well as academic level. This work has produced considerable improvements in routine diagnostic procedures for the diagnosis of chest tumors over the previous standard. Ultrasound-assisted TTNA and flexible bronchoscopy with TBNA as the leading sampling method make up more than half of all diagnostic procedures of chest tumors in our bronchoscopy theaters to date. Specialist registrars are trained to perform these procedures. Without embarking on major investments for new technologies, our diagnostic yield has been considerably increased by the routine

addition of rapid on-site evaluation of specimens. In turn, procedures yielding a sample suitable for ROSE have been developed and promoted. One more study was completed while awaiting review and publication of the papers covered in this thesis. The study dealt with evaluating different staining modalities for rapid on-site evaluation of transbronchial needle aspirates. The abstract was presented at the European Respiratory Conference 2006. As this study is not a formal part of this thesis it is presented in the Appendix only in abstract form.

7.3. Impact of this work beyond our own practice

The results of this work have been met with considerable interest on an international level. There seems to be a need for additional information and more in-depth instruction regarding the optimal use of conventional diagnostic tools. The candidate has received invitations to speak at and chair sessions about interventional bronchoscopy. A regular exchange of research fellows interested in interventional procedures has been established. Owing to our increasing international reputation it has been possible to obtain sponsorships for both diagnostic instruments as well as consumables, which are currently provided free of charge from several institutions situated in Switzerland, Germany and the Netherlands.

7.4. Outlook

Indications and technique of 22G and 21G TBNA are well documented. These small-diameter transbronchial needles are safe, relatively easy to handle and harvest high-quality cytological specimens. In comparison, little research has gone into larger bore TBNA (20G, 19G). Such needles are technically more challenging to use and there is a perceived (but as yet unproven) increased risk of bleeding. Cytology alone is sometimes not sufficient to diagnose non-carcinomatous mediastinal lymphadenopathy. This problem typically arises in cases of sarcoidosis, tuberculosis (TB) and lymphoma, where material suitable for histological preparation would be preferable. Large bore needles designed to harvest histological samples are available on the market, but standard procedures for their use are lacking. Such needles typically harvest a mixture of loose cells and fragments of tissue. These fragments are often of an intermediate size, which is not quite large enough for routine histological preparation and still too big for preparing a thin cytological smear. The ideal

preparation method for large-bore TBNA samples should combine the advantages of cytological and histological preparation. Large-bore TBNA offers research opportunities into preparation methods, clinical indications and procedural techniques.

Another area in need of research is the diagnosis of TB in mediastinal lymph nodes. TB presenting as mediastinal lymphadenopathy is becoming more frequent with the rising proportion of patients with retroviral disease. Children often present with intrathoracic lymphadenopathy, which might be easily diagnosed with TBNA. How to best prepare TBNA samples for the diagnosis of TB is not entirely clear. It has become our routine to rinse the fresh sample directly into a Mycobacterium Growth Indicator Tube (MGIT) without prior decontamination. This appears to yield good results but still needs formal validation. Such research could start with cervical lymphadenopathy and standard manual aspiration techniques. Different methods for TB culture could be validated on such samples. There is an abundance of cervical lymph node aspirates available from patients with TB, carcinoma or other infective diseases. Once the technique is established on cervical aspirates, it could be tested further on TBNA samples. As a further topic, the different immune responses in cervical or mediastinal lymph node tuberculosis could be studied on such samples.

The value of transthoracic ultrasound for chest physicians is still incompletely researched. One area of interest is the potential role of ultrasound guided aspiration in parenchymal inflammatory processes, such as the lung infiltrates observed in lobar pneumonia or acute respiratory distress syndrome (ARDS). Little is known about the pleural morphology in cases with exudative pleural effusion. Subtle changes are visible with transthoracic ultrasound, but the diagnostic value of these observations is unknown. It is also plausible that “blind” pleural biopsies could be directed towards areas with ultrasonographic pathology, thus increasing the diagnostic yield.

8. Acknowledgments

This work would have been impossible without the generous contributions of many. I would particularly like to express my thanks to:

Prof Chris Bolliger for academic, financial and moral support throughout this work.

Prof Colleen Wright, Dr Mercia Louw, Dr Karen Brundyn, Dr Pawel Schubert for the academic input, the outstanding flexibility and the many hours spent in interventional theaters.

Prof G Walzl, Dr J Theron, Dr M Schuurmans, Dr C Koegelenberg and many others who performed procedures or assisted when necessary.

Dr Coenie Koegelenberg for translating the abstract into Afrikaans.

Martin Kidd, Center for Statistical Consultation, for statistical support.

The staff of A5 bronchoscopy theatre, and the patients and the administration of Tygerberg Hospital, where all procedures took place.

The University of Stellenbosch for providing a postdoctoral research grant, and the Department of Internal Medicine for providing a place to work.

My wife Annette for assistance with keeping track of thousands of pages of data.

Friends and colleagues from Switzerland, The Netherlands and Germany for supporting the work with donations of consumable items.

The South African Thoracic Society, the European Respiratory Society, The American Thoracic Society, the Thoracic Society of Australia & New Zealand, for travel support and for providing opportunities to present the data generated with this work.

9. References

1. Spiro SG, Silvestri GA. One hundred years of lung cancer. Am J Respir Crit Care Med 2005;172:523-529.

2. Osann KE. Epidemiology of lung cancer. Curr Opin Pulm Med 1998;4:198-204.

3. Mountain CF. Revisions in the International System for Staging Lung Cancer. Chest 1997;111:1710-1717.

4. Testa JR, Pass HI, Carbone M. Benign and malignant mesothelioma. In: Principles and Practice of Oncology, 6th Ed; 2001.

5. Mullen B, Richardson JD. Primary anterior mediastinal tumors in children and adults. Ann Thorac Surg 1986;42:338-345.

6. Silverman NA, Sabiston DCJ. Mediastinal masses. Surg Clin North Am 1980;60.

7. Knapp RH, Hart RD, Payne WS. Malignant germ cell tumors of the mediastinum. J Thorac Cardiovasc Surg 1985;89:82.

8. Mazzone P, Jain P, Arroliga AC, Matthay RA. Bronchoscopy and needle biopsy techniques for diagnosis and staging of lung cancer. Clin Chest Med 2002;23:137-158.

9. Wang KP, Selcuk ZT, Erozan Y. Transbronchial needle aspiration for cytology specimens. Monaldi Arch Chest Dis 1994;49:265-267.

10. Utz JP, Patel AM, Edell ES. The role of transcarinal needle aspiration in the staging of bronchogenic carcinoma. Chest 1993;104:1012-1016.

11. Bilaceroglu S , Perim K , Gunel O , Cagirici U , Buyuksirin M . Combining transbronchial aspiration with endobronchial and transbronchial biopsy in sarcoidosis. Monaldi Arch Chest Dis 1999 ;54:217-223 .

12. Harkin TJ, Ciotoli C, Addrizzo-Harris DJ, Naidich DP, Jagirdar J, Rom WN. Transbronchial needle aspiration (TBNA) in patients infected with HIV. Am J Respir Crit Care Med 1998;157:1913-1918.

13. Geraghty PR, Kee ST, McFarlane G, Razavi MK, Sze DY, Dake MD. CT-guided transthoracic needle aspiration biopsy of pulmonary nodules: needle size and pneumothorax rate. Radiology 2003;229:475-481.

14. Koh DM, Burke S, Davies N, Padley SP. Transthoracic US of the Chest: Clinical Uses and Applications. Radiographics 2002;22:E1.

15. Diacon AH, Theron J, Bolliger CT. Transthoracic ultrasound for the pulmonologist. Curr Opin Pulm Med 2005;11:307-312.

16. Minai OA, Dasgupta A, Mehta AC. Transbronchial needle aspiration of central and peripheral lesions. In: Bolliger CT, Mathur PN, editors. Interventional bronchoscopy. Basel, Switzerland: Karger; 2000. p 66-79.

17. Davenport RD. Rapid on-site evaluation of transbronchial aspirates. Chest 1990;98:59-61.

18. Baram D, Garcia RB, Richman PS. Impact of rapid on-site cytologic evaluation during transbronchial needle aspiration. Chest 2005;128:869-875.

19. Diacon AH, Schuurmans MM, Theron J, Schubert PT, Wright CA, Bolliger CT. Safety and yield of ultrasound-assisted transthoracic biopsy performed by pulmonologists. Respiration 2004;71:519-522.

20. Diacon AH, Schuurmans MM, Theron J, Louw M, Wright CA, Brundyn K, Bolliger CT. Utility of rapid on-site evaluation of transbronchial needle aspirates. Respiration 2005;72:182-188.

21. Diacon AH, Schuurmans MM, Theron J, Brundyn K, Louw M, Wright CA, Bolliger CT. Transbronchial needle aspirates: comparison of two preparation methods. Chest 2005;127:2015-2018.

22. Diacon AH, Schuurmans MM, Theron J, Brundyn K, Louw M, Wright CA, Bolliger CT. Transbronchial needle aspirates: how many passes per target site? Eur Respir J 2007;29:112-116.

23. Diacon AH, Theron J, Schubert P, Brundyn K, Louw M, Wright CA, Bolliger CT.

Ultrasound-assisted transthoracic biopsy: fine-needle aspiration or cutting-needle biopsy? Eur Respir J 2007;29:357-362.

24. Patel NM, Pohlman A, Husain A, Noth I, Hall JB, Kress JP. Conventional transbronchial needle aspiration decreases the rate of surgical sampling of intrathoracic lymphadenopathy. Chest 2007;131:773-778.

25. Harrow EM, Abi-Saleh W, Blum J, Harkin T, Gasparini S, Addrizzo-Harris DJ, Arroliga AC, Wight G, Mehta AC. The utility of transbronchial needle aspiration in the staging of bronchogenic carcinoma. Am J Respir Crit Care Med 2000;161:601-607.

26. Holty JE, Kuschner WG, Gould MK. Accuracy of transbronchial needle aspiration for mediastinal staging of non-small cell lung cancer: a meta-analysis. Thorax 2005;60:949-955.

27. Toloza EM, Harpole L, Detterbeck F, McCrory DC. Invasive staging of non-small cell lung cancer: a review of the current evidence. Chest 2003;123:157S-166S.

28. Herth FJ, Ernst A, Eberhardt R, Vilmann P, Dienemann H, Krasnik M. Endobronchial ultrasound-guided transbronchial needle aspiration of lymph nodes in the radiologically normal mediastinum. Eur Respir J 2006.

29. Gasparini S, Silvestri GA. Usefulness of transbronchial needle aspiration in evaluating patients with lung cancer. Thorax 2005;60:890-891.

30. Rong F, Cui B. CT scan directed transbronchial needle aspiration biopsy for mediastinal nodes. Chest 1998;114:36-39.

31. Herth F , Becker HD , Ernst A . Conventional vs endobronchial ultrasound-guided transbronchial needle aspiration: a randomized trial. Chest 2004 ;125:322-325 .

32. Yasufuku K, Chiyo M, Sekine Y, Chhajed PN, Shibuya K, Iizasa T, Fujisawa T. Real-time endobronchial ultrasound-guided transbronchial needle aspiration of mediastinal and hilar lymph nodes. Chest 2004;126:122-128.

33. Yasufuku K, Chiyo M, Koh E, Moriya Y, Iyoda A, Sekine Y, Shibuya K, Iizasa T,

Fujisawa T. Endobronchial ultrasound guided transbronchial needle aspiration for staging of lung cancer. Lung Cancer 2005;50:347-354.

34. Herth FJ, Eberhardt R, Vilmann P, Krasnik M, Ernst A. Real-time endobronchial ultrasound guided transbronchial needle aspiration for sampling mediastinal lymph nodes. Thorax 2006;61:795-798.

35. Gildea TR, Mazzone PJ, Karnak D, Meziane M, Mehta AC. Electromagnetic navigation diagnostic bronchoscopy: a prospective study. Am J Respir Crit Care Med 2006;174:982-989.

36. Herth FJ , Becker HD . Transthoracic ultrasound . Respiration 2003;70:87-94 .

37. Mayo PH, Doelken P. Pleural ultrasonography. Clin Chest Med 2006;27:215-227.

38. Evans AL , Gleeson FV . Radiology in pleural disease: state of the art . Respirology 2004;9:300-312 .

39. Herth FJ, Eberhardt R, Ernst A, Becker HD. Diagnosis of pneumothorax by means of transthoracic ultrasound: a prospective trial. Eur Respir J 2004;24:S491.

10. List of appendices

A. ***Safety and yield of ultrasound-assisted transthoracic biopsy performed by pulmonologists***

Respiration 2004;71:519-22

B. ***Editorial to study A***

Respiration 2004;71:448-449

C. ***Utility of rapid on-site evaluation of transbronchial needle aspirates***

Respiration 2005;72:182-8

D. ***Editorial to study C***

Respiration 2004;71:448-449

E. ***Transbronchial needle aspirates: comparison of two preparation methods***

Chest 2005;127:2015-8

F. ***Transbronchial needle aspirates: how many passes per target site?***

European Respiratory Journal 2007;29:112-6

G. ***Ultrasound assisted transthoracic biopsy: fine needle aspiration or cutting needle biopsy?***

European Respiratory Journal 2007;29:357-62

H. ***Randomised comparison of two staining strategies for rapid on-site analysis of transbronchial needle aspirates.***

Oral presentation at European Respiratory Society Meeting 2006, Munich. European Respiratory Journal 2006;28:535s

I. ***List of other publications generated with this thesis***

Appendix A

Safety and yield of ultrasound-assisted transthoracic biopsy performed by pulmonologists

Respiration 2004;71:519-22

Interventional Pulmonology

Respiration

Respiration 2004;71:519–522
DOI: 10.1159/000080638

Received: September 8, 2003
Accepted after revision: February 2, 2004

Safety and Yield of Ultrasound-Assisted Transthoracic Biopsy Performed by Pulmonologists

A.H. Diacon[a] M.M. Schuurmans[a] J. Theron[a] P.T. Schubert[b] C.A. Wright[b] C.T. Bolliger[a]

Departments of [a]Internal Medicine and [b]Anatomical Pathology, Tygerberg Academic Hospital and Stellenbosch University, Cape Town, South Africa

For editorial comment see p. 448

Key Words
Lung, biopsy · Mediastinum, biopsy · Mesothelioma · Pleura, biopsy · Ultrasonography

Abstract

Background: Transthoracic ultrasound (US) has gained popularity as a tool for visualizing pleural effusions and assisting thoracentesis or chest drain placement. In the absence of effusion, US just as well demonstrates solid masses involving or abutting the pleura, yet biopsy of such lesions is not widely performed by chest physicians. ***Objective:*** To assess the feasibility and the safety of US-assisted cutting needle biopsy performed by chest physicians in routine practice. ***Methods:*** Lesions involving or abutting the pleura ≥20 mm in diameter on US were sampled with a 14-gauge cutting needle under local anesthesia. Biopsy site, needle direction and depth of penetration were determined with US. The procedure was performed without direct US guidance in 'free-hand' technique. ***Results:*** Ninety-one patients underwent 96 cutting-needle biopsies for suspected peripheral lung tumors (n = 44, 46%), pleural-based (n = 39, 41%), mediastinal (n = 10, 10%), or chest wall lesions (n = 3, 3%), which were single in 71%, multiple in 6% and diffuse in 23%. Sensitivity for malignant neoplasms (n = 65) was 85.5% and 100% for mesothelioma (n = 10). Pneumothorax occurred in 4%. ***Conclusions:*** US-assisted cutting-needle biopsy of lesions ≥20 mm in diameter is safe in the hands of pulmonologists. The yield for neoplastic disease including mesothelioma is high.

Introduction

Modern ultrasound (US) units are mobile, light-weight and affordable. In recent years, chest physicians have come to appreciate US as a quick and versatile bedside aid for assessing pleural effusions and assisting thoracentesis or chest drain placement. US also demonstrates pleural-based masses and lung tumors in the absence of a pleural effusion, provided they are involving or abutting the pleura [1, 2]. Such lesions are suitable for cutting-needle biopsy after identification of a puncture site with US, which has distinct potential advantages in this setting. Firstly, large vessels and aerated lung parenchyma can easily be detected with US, which minimizes the risk of

KARGER

Fax + 41 61 306 12 34
E-Mail karger@karger.ch
www.karger.com

0025–7931/04/0715–0519$21.00/0

Accessible online at:
www.karger.com/res

Andreas H. Diacon, MD
Department of Internal Medicine
University of Stellenbosch, PO Box 19063
7505 Tygerberg (South Africa)
Tel. +27 21 938 9556, Fax +27 21 931 7442, E-Mail ahd@sun.ac.za

pneumothorax and improves safety. Secondly, US can be performed at the bedside and in any body position allowing for swift procedures with minimal distress even in patients in poor general condition. Thirdly, the integration of US into a 'low-tech' procedure by chest physicians can reduce the need for more expensive radiological or surgical biopsy. This study prospectively assessed the safety and yield of US-assisted transthoracic cutting-needle biopsies performed by pulmonologists in routine practice.

Patients and Methods

Patient Selection

Patients referred to the Lung Unit of Tygerberg Hospital (tertiary academic center, catchment area 1.5 million) with pleural-based tumors on chest radiography or computed tomography (CT) were candidates for the study. Patients with pleural effusions were not enrolled but further evaluated with pleural fluid cytology or thoracoscopy. Lesions invisible or too small for biopsy on US were further investigated with appropriate means. All patients signed informed consent, and ethical approval from the institutional review board was obtained.

Biopsy Procedure

Lesions extending at least 20 mm in any accessible dimension on US (Toshiba Sonolayer L SAL 77A) were selected. A consultant respiratory physician or a registrar under supervision performed sonography and biopsy in a bronchoscopy suite without specialist radiology support. Preferred patient position for the biopsy was sitting upright on a stretcher with arms resting on a bedside table, but supine or lateral decubitus positions were also employed according to tumor location and patient preference. The operator determined the biopsy site using a standard 3.75-MHz sector probe and memorized direction and depth of penetration. As we aimed for a simple and straightforward procedure, the biopsy was performed in 'free-hand' technique without direct US observation. Care was taken to avoid intercostal and mammarian arteries. We used a manually operated 14-gauge Tru-cut biopsy needle with a specimen notch of 20 mm (Allegiance, Chateaubriand, France). Under local anesthesia, two or more passes were performed until macroscopically satisfactory material was harvested, which was routinely processed for histological evaluation. Additional tests were requested at the discretion of the physician performing the biopsy. Directly after the procedure, the site was re-examined with US. A follow-up chest radiography was obtained if the pre- and post-biopsy sonar finding differed and at the discretion of the physician. All patients were observed for at least 2 h before discharge.

Assessments and Measurements

Lesions were classified on radiological appearance for the most likely origin as 'pleural' (pleural based, blunt angle to the lung), 'pulmonary' (lesion with center in the lung, acute angle to the pleura), 'chest wall' (lesions centered in the chest wall with pleural involvement), or 'mediastinal' (lesions predominantly located in the anterior mediastinum with extension to the pleura), and for distribution as 'single', 'multiple', or 'diffuse'. A history of a recent non-diagnostic bronchoscopy was noted as a marker for a peripheral and circumscribed lesion. As an indication for locally advanced disease, we recorded the presence of superior vena cava syndrome or signs of direct spread into nervous tissue (nerve roots, spinal cord, brachial plexus), which both mean surgical non-resectability in the setting of non-small cell lung cancer.

Scoring

Histology was classified as 'diagnostic' or 'non-diagnostic' (normal tissue, not representative tissue, or representative but necrotic tissue). Two pathologists independently reviewed all specimens. For calculation of sensitivity and specificity, histological diagnosis of a specific neoplastic disease was accepted as true positive for malignancy. True negative for malignancy were lesions with non-malignant histology if confirmed by surgical biopsy, microbiological culture or radiological follow-up documenting a stable or receding lesion over at least 6 months. Likewise, cases with non-diagnostic histology but stable or receding lesions on follow-up were rated true negative. All other non-diagnostic biopsies were considered false negative.

Statistical Analysis

As this was mainly an observational study, no formal sample size calculation was performed. As we considered the yield for mesothelioma a pivotal factor for the practical value of the procedure, we arbitrarily enrolled consecutive patients until 10 cases of mesothelioma had been diagnosed. To detect a potential 'learning effect' during the study, the incidence of complications in the first and second half of the series was compared with a χ^2 test.

Results

Patients and Lesions

Over a 24-month period, 91 patients underwent 96 biopsies for 44 suspected peripheral lung tumors (46%), 39 pleural-based (41%), 10 mediastinal (10%) and 3 chest wall lesions (3%), which were single in 71%, multiple in 6% and diffuse in 23%. Seventy-four percent were male, mean age was 56 years (range: 18–80), and 38% were outpatients. Most lesions presented either as single lung lesion extending to the pleura (71%) or as diffuse pleural thickening (23%). A chest radiography was available in all and a chest CT scan in only 65 patients (68%). A peripheral lung lesion undiagnosed on bronchoscopy was noted in 24 patients (25%) and locally advanced disease with involvement of the superior vena cava or nervous tissue in 27 (28%). Mean maximal lesion thickness perpendicular to the pleura was 54 mm (range: 20–100 mm).

Procedures and Adverse Events

Mean procedure time was 25 min (range: 10–85 min). The biopsies were generally well tolerated. A pneumothorax was observed in 3 patients. Two of these had a lung lesion <30 mm in diameter which was not adherent to the

Table 1. Patients and histological diagnosis

Diagnosis	All patients (n = 91)		Pleural lesions (n = 39)	
	n	%	n	%
Diagnostic biopsies	76	84	33	85
Malignant lesions	65	71	24	62
Small cell lung cancer	14	15	4	10
Non-small cell lung cancer	35	38	8	21
Mesothelioma	10	11	10	26
Metastasis[a]	4	4	1	3
Sarcoma, myeloma	2	2	1	3
Benign lesions	11	12	19	49
Fibrosis	8	9	8	21
Solitary fibrous tumor	1	1	1	3
Involuted echinococcus cyst	1	1	0	0
Tuberculoma	1	1	0	0
Non-diagnostic biopsies	15	16	6	15
Diagnosis made surgically[b]	4	4	3	8
Lesion resolved on follow-up	4	4	3	8
Undiagnosed	7	8	0	0

[a] Germ cell tumor (n = 2), renal carcinoma (n = 2).
[b] Fibrosarcoma, hemangio-endothelioma, large cell lung cancer, solitary fibrous tumor (n = 1 each).

parietal pleura. Although diagnostic material was harvested in both lesions, movement with respiration probably led to incidental puncture of lung tissue. The other patient had a lesion of 60 mm in diameter which was centrally necrotic. A biopsy of the very periphery of the lesion was diagnostic for squamous cell carcinoma, but led to a small pneumothorax. An additional patient with severe chronic obstructive pulmonary disease reported increased dyspnea immediately after the procedure, and a small-bore chest drain was prophylactically inserted in theater. No air leak was later found, however, and the dyspnea subsided on inhalation of bronchodilators. Although it remained unclear whether this patient had a biopsy-induced pneumothorax, we calculated the rate of pneumothorax as 4% (including this patient), with a pleural drain inserted in 2% of all procedures. One case with a superficial wound hemorrhage required a single skin suture (1%). This patients had obstruction of the superior vena cava, which predisposed to bleeding from collateral veins situated in the chest wall. Minor events were transient pain requiring medication (n = 5), mild and transitory hemoptysis (n = 2) and vasovagal reaction (n = 2). Nine complications occurred in the first half versus five in the second half of the series, but this difference was not statistically significant (p = 0.22).

Specimens and Histology

Diagnostic specimens were obtained in 76 patients (84%, table 1). Most malignant lesions were non-small cell lung cancers. Detection of malignancy had a sensitivity of 85.5% and a specificity of 100%, with a positive and negative predictive value of 100 and 57.7%, respectively. Thirty-eight out of 44 lesions <50 mm (86%) and 38 out of 47 lesions ≥ 50 mm (81%) were diagnostic. Lesion size did not correlate with positive histology (not significant, χ^2 test). Seven (8%) of 15 patients (16%) with initial non-diagnostic biopsies remained undiagnosed. These patients were suffering from advanced neoplastic disease, and further investigations were not undertaken due to patient preference or lack of potential change of outcome. However, all these cases had clinical features that were not compatible with mesothelioma. We therefore determine the yield of cutting-needle biopsy for malignant mesothelioma in this series as 100%. All mesotheliomas could be diagnosed on the first biopsy procedure.

Discussion

This prospective study shows that US-assisted cutting-needle biopsy performed by a pulmonologist is safe and effective in lesions ≥20 mm in diameter abutting or involving the pleura. In a spectrum of disease representative for general practice, the sensitivity of the procedure for neoplastic disease was 85.5% and 100% for malignant mesothelioma. The intervention was safe with a rate of only 2% of pneumothorax requiring drainage. US is already widely used by pulmonologists as a bedside aid for placing chest tubes and performing pleural interventions in immobilized or ventilated patients [1]. In the dispute about the general relevance of US for the pulmonologist, this report supports the growing body of evidence that US is set to integrate with the pulmonologist's armamentarium in the future.

Pleural fluid cytology is increasingly preferred over traditional closed needle biopsy for evaluating malignant pleural effusions. Moreover, it has recently been shown in cases with cytology-negative effusions that standard Abrams needle biopsy has a lower sensitivity for pleural neoplastic disease than CT-guided cutting-needle biopsy (47 vs. 87%, respectively) [3]. This excellent result was achieved in cases with minimal pleural thickening of mainly <5 mm, which is out of range for the method studied here. However, the yield of 85.5% in the present study suggests that 'low-tech' US assistance might substitute CT guidance for lesions ≥20 mm in diameter, irrespective of the presence of a pleural effusion. Moreover, physician-operated US is far more accessible than CT in many peripheral health-care facilities, and a simple and low-cost diagnostic technique is particularly welcome in regions with high asbestos exposure. Our high yield for mesothelioma is corroborated by other recent reports [3–5]. All these studies share the use of a cutting biopsy device. It seems, therefore, that transsectional cutting biopsy specimens are better suited for the histological diagnosis of mesothelioma than Abrams needle biopsies, which are small, superficial and prone to crush artifacts.

The low rate of adverse events in the present study confirms that the lesion size and needle type chosen for this study were adequate. US has the inherent safety advantage of visualizing only lesions not shielded by air-containing tissue. Aerated lung is therefore not transversed with the biopsy device, which makes pneumothorax and air embolus unlikely when a closed cutting-needle system is used. Yield and safety of the procedure might be further improved with direct US guidance, which requires an additional skilled person to operate the sonar transducer while the biopsy is taken. This has recently been recommended for biopsy of small lesions [1]. Further, 20- or 21-gauge fine-needle aspiration (FNA) for direct on-site assessment by a cytopathologist could obviate the need for a histological sample. Pleural FNA has not been extensively studied, however, and the type of lesion suitable for FNA needs further evaluation. In summary, transthoracic US-assisted cutting-needle biopsy is an excellent first-line diagnostic tool for pleural-based lesions ≥20 mm in diameter. It is a quick, low-cost, safe and well-tolerated tool in the hands of pulmonologists and has a high sensitivity for pleural-based malignancies.

Acknowledgment

The authors would like to thank the Voluntary Academic Association Basel, Switzerland, and the Holland Stellenbosch Medical Foundation, The Netherlands, for their support.

References

1 Beckh S, Bolcskei PL, Lessnau KD: Real-time chest ultrasonography: A comprehensive review for the pulmonologist. Chest 2002;122:1759–1773.
2 Herth FJF, Becker HD: Transthoracic ultrasound. Respiration 2003;70:87–94.
3 Maskell NA, Gleeson FA, Davies RJO: Standard pleural biopsy versus CT-guided cutting-needle biopsy for diagnosis of malignant disease in pleural effusions: A randomised controlled trial. Lancet 2003;361:1326–1331.
4 Heilo A, Stenwig AE, Solheim OP: Malignant pleural mesothelioma: US-guided histologic core-needle biopsy. Radiology 1999;211:657–659.
5 Adams RF, Gray W, Davies RJ, Gleeson FV: Percutaneous image-guided cutting needle biopsy of the pleura in the diagnosis of malignant mesothelioma. Chest 2001;120:1798–1802.

Appendix B

Editorial to study A

Respiration 2004;71:448-449

Editorial

Respiration

Respiration 2004;71:448–449
DOI: 10.1159/000080627

Progress through Sound and Vision

A. Scarsbrook F.V. Gleeson

Department of Radiology, Churchill Hospital, Oxford, UK

In this issue of *Respiration*, Diacon et al. [1] report their work on ultrasound-assisted transthoracic cutting needle biopsy performed by pulmonologists. Their results are impressive with a low complication rate (4% developed pneumothorax) and high yield of diagnostic specimens (85.5%). These figures are comparable with previous series reported by interventional or chest radiologists [2, 3]. Although a 0% pneumothorax rate has been reported for lesions abutting the pleural surface [4], the biopsies in their study were not performed under direct ultrasound guidance and the patients were carefully selected, with all lesions biopsied involving or abutting the pleura and measuring at least 20 mm in diameter. Other workers have shown that cutting needle biopsy of small lesions can also be safely undertaken with an excellent diagnostic yield [3]. However, direct ultrasound guidance is necessary for smaller lesions to avoid inadvertent puncture of aerated lung. Ultrasound-guided biopsy of small lesions is technically more difficult and requires a greater degree of training and experience than for lesions greater than 3 cm, which, as has been shown by Diacon et al. [1] can be safely performed 'freehand' without direct visualisation.

The paper raises a number of interesting points: ultrasound examinations and guided biopsies have traditionally been performed by radiologists. With the advent of high quality, portable and affordable ultrasound machines there has been a rapid increase in ultrasound performed by non-radiologists. This has significant advantages for patient management: procedures that previously required transport to the radiology department can quickly and easily be performed in the outpatient clinic or at the bedside [5]. Even though ultrasound is operator dependent, basic skills such as the detection of a pleural effusion, for example, can be learnt in a short period, although a prior report has shown that involvement in 200 or fewer cases during the training period was not sufficient for physicians to develop acceptable levels of competence in sonography [6]. At the other end of the spectrum, the skills needed to perform more complex ultrasound-guided biopsy of small focal lesions with a reliable yield and low complication rate may require a longer period of training.

Whilst the pulmonologists of tomorrow fortunate enough to be trained by Diacon et al. [1] will undoubtably receive excellent training from a team with a track record that speaks for itself, there are important factors to be considered before other centres decide to adopt a similar practice. Although for the most part it does not really matter who performs transthoracic biopsies as long as effective training and clinical governance programs are in place so that the performance figures reported in the studies by Diacon et al. [1] and others [2, 3] can be maintained, it is important particularly in the setting of lung and pleural malignancy, that there is multidisciplinary team involvement in order to decide the most appropriate course for initial diagnosis and subsequent treatment. For example in the case of a suspected lung cancer there may

KARGER

Fax + 41 61 306 12 34
E-Mail karger@karger.ch
www.karger.com

0025–7931/04/0715–0448$21.00/0

Accessible online at:
www.karger.com/res

Dr F.V. Gleeson
Department of Radiology, Churchill Hospital
Old Road
Headington Oxford OX3 7LJ (UK)
E-Mail samantha.attwood@orh.nhs.uk

be many different ways in which a histological diagnosis may be obtained, each performed by a different specialist, e.g. transbronchial biopsy by the pulmonologist, percutaneous biopsy by the radiologist, video-assisted thoracoscopy and biopsy by the thoracic surgeon. Each method may have its own risks and benefits and the best approach is undoubtably reached by discussion between specialists at a regular meeting.

In summary Diacon et al. [1] should be congratulated on a well-constructed and useful paper which has important implications for the future.

Dr. Andrew Scarsbrook
Dr. Fergus Gleeson

References

1 Diacon AH, Schuurmans MM, Theron J, Schubert PT, Wright CA, Bolliger CT: Safety and yield of ultrasound-assisted transthoracic biopsy performed by pulmonologists. Respiration 2004;71:519–522.
2 Sheth S, Hamper UM, Stanley DB, Wheeler JH, Smith PA: Ultrasound guidance for thoracic biopsy: A valuable alternative to CT. Radiology 1999;210:721–726.
3 Liao WY, Chen MZ, Chang YL, Wu HD, Yu CJ, Kuo PH, Yang PC: US-guided transthoracic cutting biopsy for peripheral thoracic lesions less than 3 cm in diameter. Radiology 2000; 217:685–691.
4 Haramati LB, Austin JHM: Complications after CT-guided needle biopsy through aerated versus nonaerated lung. Radiology 1991;181: 778.
5 Doelken P, Strange C: Chest ultrasound for 'Dummies'. Chest 2003;123:332–333.
6 Hertzberg BS, Kliewer MA, Bowie JD, Carroll BA, Delong DH, Gray L, Nelson RC: Physician training requirements in sonography: How many cases are needed for competence? AJR 2000;174:1221–1227.

Appendix C

Utility of rapid on-site evaluation of transbronchial needle aspirates

Respiration 2005;72:182-8

Interventional Pulmonology

Respiration

Respiration 2005;72:182–188
DOI: 10.1159/000084050

Received: June 30, 2004
Accepted after revision: August 30, 2004

Utility of Rapid On-Site Evaluation of Transbronchial Needle Aspirates

Andreas H. Diacon[a] Macé M. Schuurmans[a] Johan Theron[a] Mercia Louw[b]
Colleen A. Wright[b] Karen Brundyn[b] Chris T. Bolliger[a]

Departments of [a]Internal Medicine and [b]Anatomical Pathology, Tygerberg Academic Hospital, University of Stellenbosch, Cape Town, South Africa

For editorial comment see p. 129

Key Words
Bronchoscopy · Fine-needle biopsy · Lung neoplasms · Cytodiagnosis · Transbronchial needle aspiration

Abstract
Background: Rapid on-site evaluation has been proposed as a method to improve the yield of transbronchial needle aspiration. ***Objectives:*** This study investigated whether on-site analysis facilitates routine diagnostic bronchoscopy in terms of sampling, yield and cost. ***Methods:*** Patients with lesions accessible for transbronchial needle aspiration on computed tomography were investigated. A cytopathologist screened the needle aspirates on site for the presence of diagnostic material. The bronchoscopic sampling process was adjusted according to the results. In 90 consecutive patients with neoplastic disease (n = 70; 78%), non-neoplastic disease (n = 16; 18%) or undiagnosed lesions (n = 4; 4%) we aspirated 162 lung tumours or lymph node sites (mediastinal: 7%; tracheobronchial: 68%; other: 25%). In 90 consecutive patients with neoplastic disease (n = 70; 78%), non-neoplastic disease (n = 16; 18%) or undiagnosed lesions (n = 4; 4%) we aspirated 162 lung lesions (paratracheal tumours or lymph nodes: 7%; tracheobronchial lymph nodes: 68%; other: 25%). ***Results:*** The diagnostic yield of needle aspiration was 77 and 25% in patients with neoplastic and non-neoplastic lesions, respectively. Sampling could be terminated in 64% of patients after needle aspiration had been performed as the only diagnostic modality, and on-site analysis identified diagnostic material from the first site aspirated in 50% of patients. Only in 2 patients (2%) diagnostic aspirates were not recognized on site. On-site analysis was cost effective due to savings for disposable diagnostic tools, which exceeded the extra expense for the on-site cytology service provided. ***Conclusions:*** Rapid on-site analysis of transbronchial aspirates is a highly useful, accurate and cost-effective addition to routine diagnostic bronchoscopy.

Introduction

Transbronchial needle aspiration (TBNA) via flexible bronchoscopy (FB) is a well-established sampling tool for a variety of malignant, infectious or granulomatous lung lesions [1]. In the setting of non-surgical staging of lung cancer, TBNA has been shown to decrease the need for

KARGER

Fax +41 61 306 12 34
E-Mail karger@karger.ch
www.karger.com

0025–7931/05/0722–0182$22.00/0

Accessible online at:
www.karger.com/res

Andreas H. Diacon, MD
Department of Internal Medicine
PO Box 19063
7505 Tygerberg (South Africa)
Tel. +27 21 938 9556, Fax +27 21 931 7442, E-Mail ahd@sun.ac.za

diagnostic thoracic surgery [2–4]. TBNA is superior to all other sampling modalities in peribronchial and submucosal disease and is on par with bronchoscopic forceps biopsy in endobronchial tumours with an average diagnostic yield of 80% [4, 5]. TBNA improves the yield of FB when added to bronchial washing, brushing and forceps biopsy [5–10]. Despite all these positive aspects, however, TBNA is still underutilized [1]. This has been ascribed to lack of formal training, difficulties with needle handling, poor success rates and insufficient cytological laboratory support [4, 11].

Practical innovations to improve the usefulness of TBNA are required. Endobronchial ultrasound (EBUS) [12, 13] and fluoroscopy with computed tomography (CT) [14] for needle guidance have shown potential to increase the yield of TBNA, but their impact has been limited to date. Rapid on-site evaluation (ROSE) of transbronchial aspirates by a cytopathologist present in the bronchoscopy theatre reduces the incidence of inadequate specimens [15] and improved TBNA yield in observational studies [16, 17]. A thus far undervalued advantage of ROSE is the opportunity to adjust sampling during FB based on the results obtained. With ROSE, the cytologist can declare that diagnostic material has been harvested in sufficient quantity and quality for a provisional diagnosis as well as for all later laboratory requirements. Consequently, the pulmonologist can decide to waive further sampling taking the anatomical and clinical situation into account. Given an acceptable accuracy of ROSE, we hypothesized that this strategy would simplify and abbreviate bronchoscopic sampling. We speculated further that fewer sampling tools used and specimens submitted for analysis could compensate for the cost of the on-site cytology service. This study prospectively investigated the diagnostic yield and accuracy of ROSE-TBNA as well as its potential to simplify the diagnostic process and reduce cost.

Methods

Patients and Procedures

We included consecutive patients scheduled for routine bronchoscopy in whom a lesion on computed tomography (CT) appeared accessible with TBNA, regardless of size, location or presumed diagnosis of the lesion. FB and TBNA (needle diameter 21 or 22 gauge; Bard, Billerica, Mass., USA) were performed under topical anaesthesia (Lidocaine 1%) by operators experienced with the techniques. Sites for TBNA were chosen based on clinical tumour stage, CT scan and intrabronchial findings. TBNA was always the first diagnostic modality. For staging of suspected lung cancer the potentially highest rated nodal site was sampled first. If staging was not of concern, the most promising site for providing a diagnosis was sampled first. A complete inspection of the bronchial system was performed in all patients before or after TBNA.

Rapid On-Site Evaluation of Specimens

The material aspirated was immediately expressed onto numbered glass slides. Of each aspiration, one slide was air-dried for subsequent staining in the laboratory and one was immersed in 95% ethanol for immediate rapid Papanicolaou staining [18]. Sampling was continued during the stain, which takes 180 s to complete. The stained slide was screened by a cytopathologist, who continuously reported the findings and announced when sufficient diagnostic material had been recovered for (1) a provisional diagnosis and (2) for all ancillary tests required for its confirmation and refinement (conventional and immunochemical stains). The bronchoscopist modified or terminated the sampling process based on the information provided by the cytopathologist. If no provisional diagnosis resulted from ROSE, sampling was continued with the appropriate additional modalities (brush, forceps biopsy, bronchoalveolar lavage, and bronchial wash).

Evaluation of ROSE and TBNA

All slides were transferred to the cytopathology laboratory and further processed with a Giemsa stain [18] and with other stains or immunochemical methods if necessary. The definitive TBNA diagnosis was established by a different cytopathologist with no knowledge of the on-site diagnosis. An aspirate was considered positive when it contained diagnostic material (clumps of cells with malignant features, or distinct features of granulomatous disease with or without necrosis). The provisional diagnosis issued on-site was compared to the final clinical diagnosis established in the patient after full clinical evaluation. A diagnosis of neoplastic disease was either surgically confirmed or corroborated by observation of a compatible clinical and radiological course under palliation. Tuberculosis was confirmed with either a positive stain for acid-fast bacilli or a positive culture of *Mycobacterium tuberculosis* on a respiratory specimen. Sarcoidosis was verified with the histological presence of non-caseating granuloma and/or typical bronchoalveolar lavage. The other diagnoses were made on clinical and radiological grounds.

Time- and Cost-Effectiveness Analysis

Firstly, we established whether sampling was reduced and which sampling manoeuvers were avoided due to ROSE. For that purpose, a detailed plan for specimen collection was recorded prior to each FB for the hypothetical situation that ROSE was not available. During FB, the sampling performed and the decisions based on ROSE were documented. Afterwards, the original plan was reviewed and adjusted for unexpected intrabronchial findings. This way we established an accurate alternative scenario for sampling without ROSE. Secondly, the person-minutes spent for both scenarios were compared. We stratified for three categories of health care professionals: (1) physicians (bronchoscopist and cytopathologist), (2) technologists (theatre nurse and cytology technician) and (3) administrative staff (porter service and secretaries). The analysis included the whole sequence of specimen collection, transport, preparation, analysis and reporting, beginning with the bronchoscopy and ending with the generation of the final report. This process

Table 1. Diagnosis and yield of TBNA (n = 90)

Clinical diagnosis	Patients	Diagnostic TBNA
Neoplastic disease	70 (78)	55 (79)
Small-cell lung cancer	18 (20)	16 (89)
Non-small-cell lung cancer	49 (54)	36 (73)
Lymphoma	2 (2)	2 (100)
Metastatic tumor	1 (1)	1 (100)
Non-neoplastic disease	16 (18)	4 (25)
Tuberculosis	8 (9)	3 (38)
Sarcoid	3 (3)	1[1] (1)
Pneumonia	3 (3)	0
Silicosis	2 (2)	0
Undiagnosed	4 (4)	0
All	90 (100)	59 (66)

Values in parenthesis are percent.
[1] Non-caseating granuloma recovered with a 21-gauge cytology needle. Confirmed with a 19-gauge histology needle specimen.

Table 2. Sites and yield of TBNA overall and in patients with neoplastic disease

	Aspirated	Positive, %
All patients (n = 90)	162	52
Paratracheal node or mass[1]*	11	45
Tracheobronchial N2 or N3	110	47
Tracheobronchial right	26	54
Tracheobronchial left	15	27
Infra- or precarinal	69	49
N1 location	22	59
Other[2]	19	89
Patients with neoplastic disease (n = 70)	128	65
Paratracheal node or mass[1]	9	56
Tracheobronchial N2 or N3	81	60
Tracheobronchial right	22	64
Tracheobronchial left	11	36
Infra- or precarinal	48	65
N1 location	20	60
Other[2]	18	94

N1, N2, N3 = Lymph node locations according to ATS [19].
[1] Above tracheobronchial.
[2] Peribronchial, submucosal, endobronchial and peripheral.

was divided into multiple specific tasks, which were either directly timed or allocated a typical amount of person-minutes to be completed. On the basis of the person-minutes spent, thirdly, the salary costs of both scenarios were calculated on the basis of the 2002 local salaries. Fourthly, the costs for consumable diagnostic tools were calculated based on the 2002 local distributors' price list. Finally, a cost balance for both scenarios was created by addition of all salary and hardware costs. Time and cost factors that did not differ between the scenarios were ignored, as they did not influence the final result.

Statistical and Ethical Considerations

A sample size calculation was performed in order to detect a significant reduction in the need for forceps biopsies due to successful ROSE-TBNA. Based on published data and previous experience, we assumed that malignant disease would be encountered in 75% of patients. Of these, 40% would be diagnosed on TBNA with a 75% success rate of ROSE. Based on these conservative estimates a cohort of 85 patients would be necessary for significance in a two-tailed test of proportions ($p < 0.05$, power 0.8). The institutional ethical review board approved the study. All patients gave written informed consent.

Results

Patients, Diagnosis and TBNA

Ninety patients (76% male) with a mean age of 57 years (range: 15–88) underwent FB with ROSE-TBNA from September 2001 to February 2003. Seventy patients (78%) had malignant disease, 16 (18%) non-malignant disease and 4 (4%) remained undiagnosed (table 1). Diagnoses were established on FB (TBNA: n = 60, other modalities: n = 11), on repeat flexible or rigid bronchoscopy (n = 3), on sputum (n = 2), on surgical (n = 2) or transthoracic biopsy (n = 3), and on clinical grounds and observation on follow-up (n = 4). In total, 162 lesions were aspirated (mean per patient: 1.8, range: 1–3). These included paratracheal tumours and lymph nodes above tracheobronchial level (7%), tracheobronchial lymph node stations (68%) and peribronchial, submucosal, endobronchial or peripheral lesions below tracheobronchial level (25%). The number of ROSE-TBNA performed was 322 (mean per site: 2.1, range: 1–4; mean per patient: 3.6, range: 1–7).

Yield and Accuracy of ROSE

Overall, TBNA was diagnostic in 60 of 90 FB (67%), in 84 of 162 aspirated sites (52%) and in 124 of 322 single aspirates (39%). The yield of TBNA was higher in neoplastic than in non-neoplastic disease, higher in small-cell than in non-small-cell lung cancer and relatively poor in left tracheobronchial position (table 2). ROSE was positive for diagnostic material in 59 procedures (66%). Out of all positive single aspirates, 84% were detected by ROSE. Not detected were 20 positive aspirates from 15 patients. In 13 of these, however, ROSE identified diag-

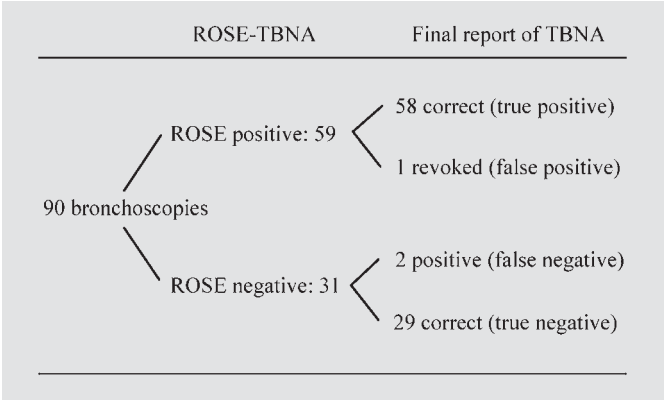

Fig. 1. Accuracy of ROSE-TBNA performed in 90 flexible bronchoscopies. Sensitivity and specificity of ROSE for detecting a diagnostic TBNA in a patient were both 96.7%; negative and positive predictive value were 93.5 and 98.3%, respectively.

Table 3. Time balance for one bronchoscopy with and without ROSE[1]

	With ROSE person-min	Without ROSE person-min	ROSE balance[2] person-min
Bronchoscopist	38	47	–9
Cytopathologist[3]	44	27	17
Theatre nurse	38	47	–9
Cytology technician	67	74	–7
Administration	8	18	–10

Rand = currency of South Africa.
[1] Average values from 90 bronchoscopies.
[2] Negative figures represent savings by ROSE.
[3] Includes 12 min for the way to the theatre and back.

nostic material on at least one other TBNA, so that only 2 patients with positive TBNA were missed by ROSE and underwent additional sampling (fig. 1). On the other hand, 2 aspirates from the same patient were false positive on ROSE, which led to inappropriate termination of the sampling process in this patient. The provisional diagnosis of non-small-cell lung cancer (NSCLC) was later confirmed with transthoracic CT-guided biopsy. Overall, sensitivity and specificity of ROSE for detection of diagnostic material in a patient with positive TBNA were both 96.7%. Negative and positive predictive values were 93.5 and 98.3%, respectively.

A specific provisional diagnosis was made in 31 out of 59 positive ROSE procedures: NSCLC subtypes (n = 19), small-cell lung cancer (SCLC; n = 11) and Hodgkin's lymphoma (n = 1). Among these, two provisional diagnoses of large cell carcinoma were amended to adenocarcinoma on the final diagnosis. Twenty-eight ROSE procedures were declared diagnostic pending further staining: Undifferentiated malignancy (n = 4), NSCLC (n = 15), SCLC or lymphoma (n = 4), granulomatous disease (n = 4) and metastatic cancer (n = 1). Except for the 1 false-positive case, all these could later be diagnosed on TBNA material. No misclassification between SCLC and NSCLC occurred with ROSE. In 1 patient, a non-caseating granuloma was recovered with a 21-gauge TBNA, and sarcoidosis was confirmed with 19-gauge TBNA needle histology specimens recovered in the same FB. This procedure was rated ROSE positive for the study.

Impact of ROSE

ROSE detected diagnostic material at the first site sampled in 50% of all procedures. Overall, 64% of FB could be terminated early because diagnosis and/or staging had been completed after TBNA. This means that sampling methods other than TBNA were only necessary in 35% of all FB performed. Moreover, ROSE-TBNA results assisted in adjusting the sampling strategy in 5 additional cases of tuberculosis or sarcoidosis, so that ROSE-TBNA had an important contribution in 71% of all FB. Compared to the alternative scenario without ROSE, the necessity to perform a forceps biopsy was significantly reduced from 65 to 18% by the employment of ROSE ($p < 0.01$, χ^2 test).

Time- and Cost-Effectiveness of ROSE

All participants, except the cytopathologist, saved time with ROSE (table 3). The bronchoscopist and theatre nurse saved time due to shorter procedure duration, the cytopathology technician prepared and screened fewer cytology and histology specimens, and less paperwork saved administrative cost. On the other hand, the cytopathologist on average spent 17 min more per patient with ROSE than for a standard laboratory-based analysis. Because the cytopathologist has a relatively high salary, the net effect on salary cost was a small loss per ROSE procedure (table 4). However, the savings for consumable tools due to ROSE outweighed the extra salary expense, even though we calculated that diagnostic brushes and biopsy forceps would be used for 3 and 7 procedures, respectively. This means that ROSE is cost-effective based on the infrastructure of our hospital. To adjust for differ-

Table 4. Expenses for one bronchoscopy with and without ROSE[1]

	Cost factor	With ROSE	Without ROSE	Cost balance[2]
	min, Rand	Rand spent	Rand spent	Rand
Salaries				
Bronchoscopist	3.32	126.1	156.0	–29.9
Cytopathologist	4.06	178.6	109.6	69
Theatre nurse	0.9	34.2	42.3	–8.1
Cytology technician	1.4	93.8	103.6	–9.8
Administration	0.4	4.0	7.2	–04
Total salaries				17.2
	Unit, Rand	Units used	Units used	Rand
Consumables[3]				
Cytology brush	44	0.18	0.72	–23.8
Biopsy forceps	32	0.2	0.77	–18.2
Total consumables				–42
Total cost				–24.8

Rand = currency of South Africa.
[1] Average values from 90 bronchoscopies.
[2] Negative figures represent savings by ROSE.
[3] Cost based on one brush being used for 3 and one forceps for 7 procedures, respectively.

ent local situations, the respective data in table 3 and 4 can be replaced with the locally valid numbers.

Discussion

This prospective study found that screening of transbronchial aspirates by a cytopathologist in theatre allowed sampling to be discontinued after aspiration of only one site in 50% of cases and in 64% of patients after TBNA of multiple sites without requiring other sampling modalities. ROSE had a sensitivity of 97% for diagnostic material on TBNA. Savings owing to fewer samples submitted for analysis and reduced use of consumables outweighed the cost for the extra service provided by cytopathology. These results were achieved in 90 consecutive patients with mixed pathology representative for routine practice.

Despite the high sensitivity of ROSE in this study, the overall yield of TBNA (60%) was lower than that reported by Diette et al. [16] (81%) and Chin et al. [17] (71%). This is probably due to selection bias, since ROSE was not systematically used in these studies. Moreover, the present study included a larger proportion of patients with non-neoplastic diseases, in which the yield of TBNA is lower. For aspirates in tracheobronchial location, however, the present study achieved 60% diagnostic TBNA compared an average yield of 42% in other studies [1, 2]. Besides possible differences in methodology, there are several plausible reasons why ROSE can improve the yield of TBNA. Firstly, negative or uncertain findings on ROSE can be addressed immediately with repeated aspirations of the same site with a slightly modified technique. This feedback-guided strategy leads to a variable number of aspirates and is indisputably better than sampling an arbitrary number of aspirates of uncertain quality [17]. Secondly, the often minute TBNA samples are handled and processed in the best possible way with ROSE, which is an often overlooked but important factor for good TBNA yield [15]. Thirdly, the availability of ROSE leads to a more frequent use of TBNA and hence practical expertise, which is likely to improve the performance of both bronchoscopists and cytopathologists [11]. Furthermore, ROSE encourages the use of TBNA not only for formal staging but also for submucosal, exophytic and peripheral lesions, which are known to have a good yield with TBNA [5].

ROSE provides the opportunity to stop sampling when the diagnostic objective has been met. To take full advantage of this option means to accept cytological aspirates

for diagnosis and classification of lung cancer also in patients with lesions potentially accessible for a forceps biopsy. This is controversial, because it may be argued that the diagnostic quality of needle aspirates is inferior to histological specimens provided by forceps biopsies. In contrast to all other sampling modalities, however, needle aspirates can be scrutinized for quality on site. Consequently, the cytopathologist can ask for more aspirates or even a forceps biopsy specimen to ensure that the material is adequate for all ancillary tests required. It has been shown that the concordance of TBNA and forceps biopsies is excellent [5], and that no single sampling method has a superior yield in FB [4]. It is widely accepted that combining conventional sampling methods (bronchial washing, brushing and endobronchial biopsy) with TBNA increases the yield of FB [4, 5, 8, 9]. The present study shows that the yield of FB can be predicted with high accuracy with ROSE. Multiple sampling methods are therefore only necessary in a minority of cases, which are best identified by a cytopathologist attending the procedure. For further management in bronchogenic carcinoma the accurate distinction of SCLC from NSCLC is of utmost importance. TBNA is the most sensitive sampling method for SCLC [20–23], while forceps biopsies are prone to crush artefacts and sometimes difficult to interpret [24]. The subclassification of NSCLC is less important for routine clinical practice and can be challenging on bronchoscopic specimens in general [4, 21, 25].

It has been held against ROSE that it is a time-consuming and inadequately remunerated procedure [4, 15, 16, 26]. In order to balance personnel and hardware cost involved, we undertook a detailed and comprehensive cost-effectiveness estimate. Our model showed that ROSE led to fewer biopsy tools used and fewer samples submitted for the cytology and pathology laboratories. Overall, reduced hardware and salary costs compensated for the time and cost of the on-site cytopathology service. We acknowledge that this analysis is not universally generalizable and factors such as hospital geography, local health care system and available expertise must be accounted for elsewhere. However, we believe that the cost of ROSE is generally overestimated. The superior access to clinical and radiological information in theatre is an advantage that might compensate the cytopathologist for the inconvenience of displacement. Very little has been written about the practical aspects of ROSE-TBNA, including which method of staining is more effective. The rapid Papanicolaou stain used in the present study has superior demonstration of nuclear morphology and hence neoplastic characteristics of an aspirate, but it takes 3 min to complete. Quicker stains, such as Diff-Quik [18], which are superior for demonstrating lymphocytes may still be evaluated. The type and size of the TBNA needle as well as the minimum level of experience of the specialists involved are as yet unknown variables for the yield of ROSE-TBNA.

EBUS to visualize the target area is a new tool set to improve the yield of TBNA [12, 27]. In addition, EBUS demonstrates the ultrastructure of the tracheobronchial wall and enables decision making about treatment modalities of early cancer of the central airways [13]. Even peripheral lesions and solitary pulmonary nodules are in reach of EBUS [28]. However, EBUS is still costly, requires considerable technical skill and is unlikely to become popular outside specialized centres in the near future. Interestingly, a study comparing EBUS-guided TBNA with conventional TBNA for mediastinal adenopathy showed an equally high yield with both methods [12]. ROSE was used for determination of specimen adequacy in that study, and it was speculated that ROSE might increase the sensitivity of standard TBNA to the same degree as EBUS. However, no studies have yet directly compared the yield of EBUS-guided and ROSE-assisted TBNA and the respective value of the methods needs further investigation.

The results of the present study demonstrate that ROSE increases the value of TBNA as a diagnostic modality. In our institution, bronchoscopists and cytopathologists alike have come to appreciate the high yield, accuracy and brevity of ROSE-assisted procedures. The patients benefit of a swift procedure and accelerated management provided by ROSE, which is now requested as part of our routine bronchoscopy service for every lesion likely to be reached with TBNA.

Acknowledgments

Bard Endoscopic Technologies, Billerica, Mass., USA, provided TBNA needles free of charge. A.H.D. was supported by a grant from the Th. & L. La Roche Foundation, Basel, Switzerland, and by a research fellowship grant of the University of Stellenbosch, South Africa.

References

1 Minai OA, Dasgupta A, Mehta AC: Transbronchial needle aspiration of central and peripheral lesions; in Bolliger CT, Mathur PN (eds): Interventional bronchoscopy. Basel, Karger, 2000, pp 66–79.

2 Harrow EM, Abi-Saleh W, Blum J, Harkin T, Gasparini S, Addrizzo-Harris DJ, Arroliga AC, Wight G, Mehta A: The utility of transbronchial needle aspiration in the staging of bronchogenic carcinoma. Am J Respir Crit Care Med 2000;161:601–607.

3 Schenk DA, Chambers SL, Derdak S, Komadina KH, Pickard JS, Strollo PJ, Lewis RE, Patefield AJ, Henderson JH, Tomski SM, Morales CF, Sterling JL, Solanki PH, Moore J: Comparison of the Wang 19-gauge and 22-gauge needles in the mediastinal staging of lung cancer. Am Rev Respir Dis 1993;147:1251–1258.

4 Mazzone P, Jain P, Arroliga AC, Matthay RA: Bronchoscopy and needle biopsy techniques for diagnosis and staging of lung cancer. Clin Chest Med 2002;23:137–158.

5 Dasgupta A, Jain P, Minai OA, Sandur S, Meli Y, Arroliga AC, Mehta AC: Utility of transbronchial needle aspiration in the diagnosis of endobronchial lesions. Chest 1999;115:1237–1241.

6 Salathe M, Soler M, Bolliger CT, Dalquen P, Perruchoud AP: Transbronchial needle aspiration in routine fiberoptic bronchoscopy. Respiration 1992;59:5–8.

7 Gasparini S, Ferretti M, Secchi EB, Baldelli S, Zuccatosta L, Gusella P: Integration of transbronchial and percutaneous approach in the diagnosis of peripheral pulmonary nodules or masses. Experience with 1,027 consecutive cases. Chest 1995;108:131–137.

8 Govert JA, Dodd LG, Kussin PS, Samuelson WM: A prospective comparison of fiberoptic transbronchial needle aspiration and bronchial biopsy for bronchoscopically visible lung carcinoma. Cancer 1999;87:129–134.

9 Bilaceroglu S, Gunel O, Cagirici U, Perim K: Comparison of endobronchial needle aspiration with forceps and brush biopsies in the diagnosis of endobronchial lung cancer. Monaldi Arch Chest Dis 1997;52:13–17.

10 Baaklini WA, Reinoso MA, Gorin AB, Sharafkaneh A, Manian P: Diagnostic yield of fiberoptic bronchoscopy in evaluating solitary pulmonary nodules. Chest 2000;117:1049–1054.

11 Haponik EF, Cappellari JO, Chin R, Adair NE, Lykens M, Alford PT, Bowton DL: Education and experience improve transbronchial needle aspiration performance. Am J Respir Crit Care Med 1995;151:1998–2002.

12 Shannon JJ, Bude RO, Orens JB, Becker FS, Whyte RI, Rubin JM, Quint LE, Martinez FJ: Endobronchial ultrasound-guided needle aspiration of mediastinal adenopathy. Am J Respir Crit Care Med 1996;153:1424–1430.

13 Falcone F, Fois F, Grosso D: Endobronchial Ultrasound. Respiration 2003;70:179–194.

14 Garpestad E, Goldberg S, Herth F, Garland R, LoCicero J, Thurer R, Ernst A: CT fluoroscopy guidance for transbronchial needle aspiration: An experience in 35 patients. Chest 2001;119:329–332.

15 Davenport RD: Rapid on-site evaluation of transbronchial aspirates. Chest 1990;98:59–61.

16 Diette GB, White P, Terry P, Jenckes M, Rosenthal D, Rubin HR: Utility of on-site cytopathology assessment for bronchoscopic evaluation of lung masses and adenopathy. Chest 2000;117:1186–1190.

17 Chin R, McCain TW, Lucia MA, Cappellari JO, Adair NE, Lovato JF, Dunagan DP, Brooks MA, Clark HP, Haponik EF: Transbronchial needle aspiration in diagnosing and staging lung cancer: How many aspirates are needed? Am J Respir Crit Care Med 2002;166:377–381.

18 Keebler KM: Cytopathology techniques; in Bibbo M (ed): Comprehensive Cytopathology, ed 2. Philadelphia, Saunders, 1997, pp 881–906.

19 Pretreatment evaluation of non-small-cell lung cancer. The American Thoracic Society and The European Respiratory Society. Am J Respir Crit Care Med 1997;156:320–332.

20 Barbazza R, Toniolo L, Pinarello A, Scapinello A, Falconieri G, DiBonito L: Accuracy of bronchial aspiration cytology in typing operable (stage I–II) pulmonary carcinomas. Diagn Cytopathol 1992;8:3–7.

21 DiBonito L, Colautti I, Patriarca S, Falconieri G, Barbazza R, Vielh P: Cytological typing of primary lung cancer: Study of 100 cases with autopsy confirmation. Diagn Cytopathol 1991;7:7–10.

22 Payne CR, Hadfield JW, Stovin PG, Barker V, Heard BE, Stark JE: Diagnostic accuracy of cytology and biopsy in primary bronchial carcinoma. J Clin Pathol 1981;34:773–778.

23 Chin R, Cappellari JO, McCain TW, Case LD, Haponik EF: Increasing use of bronchoscopic needle aspiration to diagnose small cell lung cancer. Mayo Clin Proc 2000;75:796–801.

24 Jones DF, Chin R, Cappellari JO, Haponik EF: Endobronchial needle aspiration in the diagnosis of small-cell carcinoma. Chest 1994;105:1151–1154.

25 Chuang MT, Marchevsky A, Tierstein AS, Kirschner PA, Kleinerman J: Diagnosis of lung cancer by fibreoptic bronchoscopy: Problems in the histological classification of non-small cell carcinomas. Thorax 1984;39:175–178.

26 Layfield LJ, Bentz JS, Gopez EV: Immediate on-site interpretation of fine-needle aspiration smears: A cost and compensation analysis. Cancer 2001;93:319–322.

27 Herth F, Becker HD, Ernst A: Conventional vs endobronchial ultrasound-guided transbronchial needle aspiration: A randomized trial. Chest 2004;125:322–325.

28 Shirakawa T, Imamura F, Hamamoto J, Honda I, Fukushima K, Sugimoto M, Shirkakusa T: Usefulness of endobronchial ultrasonography for transbronchial lung biopsies of peripheral lung lesions. Respiration 2004;71:260–268.

Appendix D

Editorial to study C

Respiration 2005;72:129-131

Editorial

Respiration

Respiration 2005;72:129–131
DOI: 10.1159/000084041

It Is Time for This 'ROSE' to Flower

Stefano Gasparini

Pulmonary Diseases Unit, Department of Respiratory and Allergic Diseases, Azienda Ospedali Riuniti 'Umberto 1° – G.M. Lancisi – G. Salesi', Ancona, Italy

In the last decades, needle aspiration techniques have gained ground in respiratory medicine, especially for the diagnosis and staging of lung cancer. Techniques such as percutaneous fine-needle aspiration and transbronchial needle aspiration (TBNA) have been demonstrated to be reliable sampling tools in clinical practice. Their use allows cytohistological diagnosis of malignancy and numerous benign conditions with good sensitivity and excellent specificity, avoiding more invasive surgical procedures such as mediastinoscopy, video-assisted thoracoscopic surgery or thoracotomy [1, 2].

One of the advantages of the cytological aspiration techniques is the possibility to immediately evaluate the material with rapid stain methods to define the adequacy of the sample and to obtain a preliminary diagnosis, if the cytopathologist is present in the diagnostic room. The presence of a cytopathologist during the needle aspiration procedures also ensures that the material will be treated and prepared in the best way. The results obtained by the immediate cytological assessment provides the operator with invaluable information on how to carry on with the procedure that can be stopped if the material is diagnostic, avoiding further and useless passes with the needle, thereby reducing time and risks. On the contrary, if the sample is not diagnostic, other needle passes can be performed by the operator who, on the basis of the information provided by the cytopathologist, could modify the technique of sampling or the point of punction. Furthermore, if required by the immediate assessment and deemed necessary by the cytopathologist, the operator could be invited to sample additional material for ancillary techniques, such as electron microscopy, immunocytochemistry or microbiological studies, or to repeat the sample using histology needles, if histological material is considered useful for perfecting the diagnosis.

A 'ROSE' is a flower, but it is also the acronym frequently employed to indicate Rapid On-Site Evaluation of the material obtained by needle aspiration techniques; other authors use the less romantic acronym ICA, which stands for Immediate Cytological Assessment [3].

In recent years, in an effort to improve the sensitivity and the diagnostic yield of the needle aspiration techniques, new technologies of guidance have been proposed (endobronchial ultrasound, virtual bronchoscopy, CT fluoroscopy, 3D navigation systems) [4–6], but only few papers have focused on the role of correct management, preparation and examination of the sampled material which is an essential point to achieve good results.

In 1993, analyzing data from 55 patients who underwent percutaneous fine-needle aspiration from a lung lesion suspected for cancer, Austin and Cohen [7] obtained a sensitivity of 100% in 25 cases performed with ROSE and of 80% in 30 cases where the biopsy was performed without the cytopathologist. In the same paper, the meta-analysis of the previously published data showed that the immediate cytological assessment was associated with a statistically significant increase in diagnostic accuracy compared with the procedures performed when a cytopathologist was not present. In 1998, in a series of 207 TBNA performed on 161 patients, comparing 73 aspirates using ROSE with 134 routinely processed samples, Davenport [8] showed that the percentage of specimens containing malignant cells increased from 31 to 56% and that the inadequate TBNA decreased from 56 to 18%

KARGER

Fax +41 61 306 12 34
E-Mail karger@karger.ch
www.karger.com

0025–7931/05/0722–0129$22.00/0

Accessible online at:
www.karger.com/res

Stefano Gasparini, UO di Pneumologia
Dipartimento delle Malattie Respiratorie ed Allergiche
Azienda Ospedaliero-Universitaria Ospedali Riuniti
IT–60020 Ancona (Italy)
Tel. +39 071 596 4374, Fax +39 071 596 4344, E-Mail s.gasparini@fastnet.it

when the cytopathologist was present. Likewise, in a prospective cohort study on 204 patients who underwent bronchoscopy for evaluation of lung nodules or masses and/or hilar and mediastinal lymphadenopathy, Diette et al. [9] obtained a diagnostic yield of 81% in the 81 cases performed with ROSE versus 50% in the cases without immediate cytological assessment.

Despite this evidence of diagnostic advantages, ROSE is still not a very widespread procedure, and in many institutions, the pulmonologists find several difficulties to persuade the cytopathologists to participate directly in needle aspiration activities.

There are mainly two reasons for these difficulties.

The first is due to a lack of pathologists specifically trained and dedicated to cytology in many institutions. Generally, traditional pathologists do not like to leave their offices, heading for the diagnostic rooms with a microscope. They think that this could mean a loss of time, taken away from their routine diagnostic activity. Pulmonologists should use their enthusiasm and make every effort to convince the pathologists of the diagnostic efficacy of ROSE, which could be compared in its value with the intraoperative frozen section examination activity that, on the contrary, is well spread and accepted worldwide. As underlined by Miller et al. [10] and as we have verified in our experience of more than 20 years [3], the great educational relevance of ROSE for the staff, arising from the opportunity to discuss the cases together at the time of the examination, should also be emphasized.

The second and possibly main reason why ROSE is not very widespread is that its cost effectiveness has not yet been demonstrated. Without doubt, the extra time and effort of the cytopathologist is a cost that should be considered and adequately reimbursed. The inadequate economical consideration does not encourage the cytopathologists to participate on site at the diagnostic procedures.

In this issue of *Respiration,* the paper of Diacon et al. [11] does not only focus on the diagnostic utility of ROSE during bronchoscopy using TBNA, but also on the economical aspects of the immediate cytological examination, with an accurate and detailed analysis of the costs of the material and the work of all the operators involved in the procedure, including physicians, nurses, cytology technicians and administrative staff. The costs have been calculated for bronchoscopies performed with ROSE and for an alternative scenario for sampling without ROSE. The results show that all the staff save time with ROSE, except for the cytopathologist who on average spends 17 min more per patient. However, even considering the relatively high salary of the cytopathologist, saving on consumable tools and time of the other operators allowed ROSE to save 24.8 rand (the currency of South Africa, where the study was performed) per patient. These data led the authors to conclude that ROSE is not only highly useful in increasing the diagnostic value of the TBNA procedures, but also cost effective.

As underlined by the same authors in the discussion, this conclusion is not universally generalizable and should be verified in other countries and other institutions, taking into account the different organizations of the various hospitals and the different costs that material and salaries could have. Economic analysis such as that made by Diacon et al. [11] should be encouraged, because if the cost effectiveness of ROSE will be universally confirmed, every effort will have to be made to diffuse this practice and to stimulate the different health care systems and hospital administrations to invest resources in this kind of diagnostic approach.

I hope that in the future the basic knowledge of cytopathology will be inserted in the training program of pulmonologists and will become part of their culture and qualification. Thus, the presence of a cytopathologist may no longer be necessary to perform ROSE. The pulmonologist could carry out the procedure by himself, further reducing the cost. In many cases, it could be enough to have a preliminary evaluation, demonstrating whether neoplastic cells are present in the specimen or not. Of course, the final diagnosis should remain the task of the pathologist, and nothing would be taken away from his competence and responsibility. It is incomprehensible why the pulmonologist should be able to read and interpret a CT scan, when he cannot evaluate, at least preliminarily, a cytological slide. Pulmonologists who are able to perform ROSE by themselves already exist [12], and they receive a lot of professional gratification from their practice.

At the end of this comment, let me talk about another reason that could have stimulated the enthusiasm of Diacon et al. [11] for ROSE. At the last ERS meeting in Glasgow, I had the chance to chair a session where a poster communication on ROSE was displayed by these authors. The picture showed a cytopathologist performing an immediate cytological evaluation on a microscope. Being used to a cytopathologist with a moustache, it was surprising for me to see that a wonderful, blonde woman was sitting at the microscope. ROSE is a romantic acronym: if you are lucky to have a nice woman as a cytopathologist, you could have one more reason to hope that this ROSE flowers.

References

1 Dasgupta A, Mehta AC, Wang KP: Transbronchial needle aspiration. Semin Respir Crit Care Med 1997;18:571–581.
2 Mazzone P, Jain P, Arroliga AC, Matthay RA: Bronchoscopy and needle biopsy techniques for diagnosis and staging of lung cancer. Clin Chest Med 2002;23:137–158.
3 Gasparini S, Ferretti M, Secchi EB, Baldelli S, Zuccatosta L, Gusella P: Integration of transbronchial and percutaneous approach in the diagnosis of peripheral pulmonary nodules or masses. Experience with 1027 consecutive cases. Chest 1995;108:131–137.
4 Herth F, Becker HD, Ernst A: Conventional vs endobronchial ultrasound-guided transbronchial needle aspiration: A randomized trial. Chest 2004;125:322–325.
5 White CS: Transbronchial needle aspiration: Guidance with CT fluoroscopy. Chest 2000; 118:1630–1638.
6 Becker HD: Bronchoscopy. Year 2001 and beyond. Clin Chest Med 2001;22: 225–239.
7 Austin JHM, Cohen MB: Value of having a cytopathologist present during percutaneous fine-needle aspiration biopsy of lung: Report of 55 cancer patients and metaanalysis of the literature. AJR Am J Roentgenol 1993;160:175–177.
8 Davenport RD: Rapid on-site evaluation of transbronchial aspirates. Chest 1998;98:59–61.
9 Diette GB, White P Jr, Terry P, Jenckes M, Rosenthal D, Rubin HR: Utility of on-site cytopathology assessment for bronchoscopic evaluation of lung masses and adenopathy. Chest 2000;117:1186–1190.
10 Miller DA, Carrasco CH, Katz RL, Cramer FM, Wallace S, Charnsangavej C: Fine needle aspiration biopsy: The role of immediate cytologic assessment. AJR Am J Roentgenol 1986; 147:155–158.
11 Diacon AH, Schuurmans MM, Theron J, Louw M, Wright CA, Brundyn K, Bolliger CT: Utility of rapid on-site evaluation of transbronchial needle aspirates. Respiration 2005; 72:182–188.
12 Marcianò G, Marino M: Broncologia. Endoscopia e Citologia. Milano, UTET Ed, 1993.

Appendix E

Transbronchial needle aspirates: comparison of two preparation methods

Chest 2005;127:2015-8

Transbronchial Needle Aspirates*

Comparison of Two Preparation Methods

Andreas H. Diacon, MD; Macé M. Schuurmans, MD; Johan Theron, MD; Karen Brundyn, MD; Mercia Louw, MD; Colleen A. Wright, MD; and Chris T. Bolliger, MD, PhD

Study objectives: **Transbronchial needle aspiration has evolved as a key bronchoscopic sampling method. Specimen handling and preparation are underrated yet crucial aspects of the technique. This study was designed to identify which of two widely practiced sample preparation methods has a higher yield.**
Design: **Prospective comparison of two diagnostic methods.**
Setting: **Tertiary academic hospital.**
Patients: **Consecutive patients undergoing transbronchial needle aspiration.**
Interventions: **Transbronchial aspirates were obtained pairwise. One specimen was placed directly onto a slide and smears were prepared on site (*ie*, the direct technique), and the other specimen was deposited into a vial containing 95% alcohol and further prepared in the laboratory (*ie*, the fluid technique). In total, 282 pairs of samples were aspirated from 145 target sites (paratracheal, 10 sites; tracheobronchial, 101 sites; hilar, 17 sites; endobronchial or peripheral, 17 sites).**
Measurements and results: **The measured outcome was the presence of diagnostic material at the final laboratory assessment. At least one diagnostic aspirate was obtained in 66% of 86 investigated patients (small cell lung cancer, 18 patients; non-small cell lung cancer, 47 patients; other diagnoses, 21 patients). The direct technique had a better yield overall than the fluid technique (positive aspirates, 36.2% vs 12.4%, respectively; $p < 0.01$), as well as after stratification for tumor type and for anatomic site.**
Conclusion: **The direct technique is superior to the fluid technique for the preparation of transbronchial needle aspirates.** ***(CHEST 2005; 127:2015–2018)***

Key words: bronchoscopy; cytodiagnosis; fine-needle biopsy; lung neoplasms

Abbreviations: ATS = American Thoracic Society; TBNA = transbronchial needle aspiration

Transbronchial needle aspiration (TBNA) via flexible bronchoscopy is an established sampling method for a variety of lung lesions.[1] The most important indication for TBNA is mediastinal staging of lung cancer. The lymph node stations that are crucial for treatment and prognosis, as defined by the TNM system,[2] are easily accessible with TBNA, which is cost-effective and reduces the need for exploratory surgery.[3] However, the method is still underutilized.[4] A possible reason for this is the failure to reproduce published success rates of TBNA.[5] Investigations[1,6] aiming to increase TBNA use and to improve overall success rates have shown that education and experience with the TBNA technique improve the yield. Much less is known about how the samples should be prepared after successful aspiration. In the original article by Wang et al,[7] the specimens were flushed into a container and transported as a fluid suspension to the laboratory, where they were processed further (*ie*, the fluid technique).[7] Alternatively, the specimen can be directly placed onto a slide, and immediately smeared and spray-fixed (*ie*, the direct technique).[8,9] Based on our own experience, we hypothesized that the direct technique would be superior to the fluid technique. This study was designed to clarify whether and to what degree specimen preparation affects the diagnostic yield of TBNA in routine practice.

*From the Departments of Internal Medicine (Drs. Diacon, Theron, Schuurmans, and Bolliger) and Anatomical Pathology (Drs. Brundyn, Louw, and Wright), Tygerberg Academic Hospital, University of Stellenbosch, Cape Town, South Africa.
Dr. Diacon was supported by a research fellowship grant of the University of Stellenbosch, South Africa.
Manuscript received September 2, 2004; revision accepted October 27, 2004.

Correspondence to: Andreas H. Diacon, MD, Department of Internal Medicine, PO Box 19063, 7505 Tygerberg, South Africa; e-mail: ahd@sun.ac.za

Materials and Methods

Transbronchial Sampling

Four experienced operators performed standard flexible bronchoscopy (models BF30 BF1T160; Olympus; Tokyo, Japan; Exera; Hamburg, Germany) and TBNA under topical anesthesia (1% lidocaine) and conscious sedation (midazolam IV). TBNA target sites were defined as (1) paratracheal (lymph nodes or lung lesions extending to the trachea), (2) tracheobronchial (American Thoracic Society [ATS] lymph node stations 1 to 4[10]), (3) hilar (ATS lymph node stations 7 and 11), (4) bronchial (*ie*, parabronchial, submucosal, and endobronchial), and (5) peripheral (*ie*, not visible from endobronchial). We used 21-gauge or 22-gauge cytology needles (Bard; Billerica, MA) and aspirated for 10 s in the standard fashion.[1] Only one needle type was used in a single patient. A paired sample consisted of two aspirate samples that were obtained in immediate succession and with identical technique, with the needle insertion points ideally 1 mm apart. This assured close proximity of the needle tips during aspiration. Preparation techniques were alternated after each pass. The direct technique was used for the first aspirate within a pair when cytologic support was available on-site. Otherwise, the fluid technique was used first. The sampling of pairs was completed without awaiting on-site results. At least four aspirates (two pairs) at each site were obtained.

Sample Preparation and Analysis

For the direct technique, the aspirate was immediately placed onto a glass slide, covered with a second slide, and, while exerting gentle continuous pressure, the slides were drawn apart. One of the smears was spray-fixed using commercial cytology fixative (Sangene; Cape Town, South Africa), and the other one was air-dried. For the fluid technique, the aspirate was deposited into 2 mL 95% alcohol and was processed further in the cytology laboratory in routine fashion. The fluid was centrifuged at 1,500 revolutions per minute for 10 min, and the resulting sediment was placed onto two slides, one spray-fixed and the other one air-dried. All slides were stained using standard Giemsa and rapid or standard Papanicolau methods.[11] Histochemical or immunohistochemical examination was performed when necessary on the destained Papanicolau slides.[11] For the study, the test results for an aspirate were considered to be positive when it contained diagnostic material (*ie*, adequate numbers of malignant cells or distinct features of granulomatous disease with or without necrosis). This was determined by two independent cytopathologists, who were unaware of the preparation method used and of any provisional diagnoses issued before the final assessment.

Statistical Aspects and Study Progress

The sample size was calculated for the detection of a 10% difference between the preparation methods assuming a 50% yield for the better method and an average of four sampled pairs per patient. A two-tailed test of proportions would show significance with 48 patients (power, 0.8; significance level, 95%). The first analysis showed a surprisingly low yield for the fluid method. This was thought to probably be due to insufficient material being expelled into the vials. Subsequently, the fluid method was modified by using a 50-mL syringe instead of a 20-mL syringe for aspiration and by expelling the sample with 1 mL of normal saline solution instead of air. This procedure might lead to better clearance of aspirated material out of the needle and cannot be performed with the smear method, because the fluid would wash the material off the slide. Consequently, separate needles for each technique were used, which also eliminated the problem of possible needle contamination with material retained from the previous pass. The target sample size was doubled in order to allow for the modifications to show an effect. Counts were compared with contingency tables and χ^2 tests ($p < 0.05$ [a significant difference]) using a statistical software package (StatView, version 4.0 for Macintosh; SAS Institute; Cary, NC). All patients gave written informed consent. The institutional ethics review board approved the study.

Results

Patients and Diagnosis

We prospectively included 90 consecutive patients (56 men) with a mean (± SD) age of 57 ± 15 years (age range, 16 to 88 years). Of these patients, four had to be excluded *post hoc* because faded slide labels did not allow the identification of the preparation method that had been used. In the remaining 86 patients, 282 pairs were aspirated from 145 target sites (paratracheal, 10 sites; tracheobronchial, 101 sites; hilar, 17 sites; bronchial or peripheral, 17 sites). Two thirds of patients had at least one positive finding from TBNA. A definitive cytologic diagnosis with TBNA was possible in more neoplastic than nonneoplastic lesions (Table 1). Among the neoplastic lesions, small cell lung cancer was more often identified than non-small cell lung cancer. Among the nonneoplastic lesions, only one case of sarcoidosis and one case of tuberculosis could be identified with TBNA. The direct method (49 patients; 57%) was used first more often than the fluid method (37 patients; 43%).

Yield of TBNA and Preparation Methods

The results of at least one TBNA was positive in 112 of 282 pairs of samples (39.7%) collected (Table 2). Only one of the techniques provided a positive aspirate in 30.8% of pairs (direct technique exclusively positive, 27.3%; fluid technique exclusively positive, 3.5%). Overall, the direct technique was

Table 1—*Patients, Diagnosis, and Yield of TBNA

Variables	Patients, No.	Positive TBNA, %
All patients	86	66
Neoplastic disease	68	81
Non-small cell lung cancer	47	77
Small cell lung cancer	18	89
Other neoplastic	3	100
Nonneoplastic disease	18	11
Infectious	9	11
Noninfectious	5	20
Undiagnosed	4	0

*Positive TBNA = at least one aspirate positive for diagnostic material.

Table 2—*Yield of Preparation Techniques Stratified for Anatomic Location and Histology

Variables	Pairs, No.	Pair,† % Positive	Direct Method, % Positive	Fluid Method, % Positive	p Value‡
All	282	39.7	36.2	12.4	< 0.01
Anatomic site					
Paratracheal mass or lymph node	25	44.0	44.0	8.0	0.01
Tracheobronchial lymph nodes	206	34.5	32.0	9.7	< 0.01
Infracarinal or precarinal (ATS 1, 2)	127	34.6	31.5	11.8	0.02
Right (ATS 3)	46	43.5	43.5	8.7	0.02
Left (ATS 4)	33	21.2	18.2	3	NS
Hilar lymph nodes (ATS 7, 11)	22	45.5	31.8	27.3	NS
Peribronchial, submucosal, exophytic, or peripheral	29	69.0	62.1	24.1	NS
Tumor type					
Small cell lung cancer	60	55.0	51.7	25.0	< 0.01
Non-small cell lung cancer	153	45.8	41.2	12.4	0.04

*NS = not significant.
†Pair = either direct method or fluid method, or both, positive for diagnostic material.
‡By χ^2 test for direct method vs fluid method.

significantly superior to the fluid technique (positive aspirates, 36.2% vs 12.4%, respectively; $p < 0.01$) [Table 2]. Although differences varied, stratification for tumor type and for anatomic target site did not identify a single constellation in which the fluid technique was superior. The modification of the fluid technique after 45 patients did not change the difference between the techniques (before modification, 34.8% vs 13%, respectively [$p = 0.01$]; after modification, 38% vs 11.6%, respectively [$p = 0.03$]).

Discussion

This prospective comparative study showed that directly prepared and smeared TBNA specimens provide better results than those expelled into alcohol for use in the laboratory. This finding might contribute to further improve TBNA results and to promote TBNA as a key technique in bronchoscopic sampling.

Being situated in the "gray zone" of competence between bronchoscopist and cytopathologist, the issues of preservation, transport, and preparation of samples have received very little attention in the past. The direct smear method was described as an alternative to the original fluid method soon after the introduction of TBNA into clinical practice.[7–9] To our knowledge, however, no sufficiently powered comparative study has compared the methods against each other, and several review articles[1,3,5] have not recommended a specific method for TBNA specimen preparation. The present study highlights the importance of the preparation method and its significant impact on the diagnostic yield of TBNA. We can only speculate as to the reason for the superiority of the direct method. Our study used the patients as their own control subjects, which virtually eliminated all variables other than the preparation method. Therefore, the most likely explanation lies with the loss of cellular material with the fluid method, which includes initial dilution, centrifugation, aspiration, and transfer of material onto the final slides. It seems logical to assume that both the quality and quantity of a sample may deteriorate with this multistep approach, even when performed by trained personnel. In contrast, the direct method allows the immediate transfer of cellular material onto slides and results in adequate, well-fixed slides for cytologic evaluation. A further advantage of directly smeared slides is their availability for rapid on-site examination.[12]

No unequivocal data exist on the absolute yield of the fluid method. However, its performance in this study might seem lower than expected in the eyes of those who routinely use it. One must remember that in standard clinical practice several successive aspirates are usually flushed into one single vial. This results in an additive yield of these specimens, while in the present study single samples were analyzed. Some shortcomings of the present study must also be mentioned. First, we employed no objective quantitative and qualitative measures for determining the slide contents. This means that we cannot prove our subjective impression that the number of cells available for analysis is reduced with the fluid method, while the quality of the cells is well-preserved. Second, we did not exploit all available variations of aspiration needle sizes and methods for the preparation of the fluid specimen. For example, a histologic sample obtained with a larger caliber needle prepared with the cellblock technique might provide even better results.[13] Third, it might be criticized

that we did not randomize the sequence of preparation techniques used in a paired sample. It is plausible that the second sample could have greater chances of containing diagnostic material, since some might have been retained in the needle after the first pass. However, this would not have changed the study outcome since second samples were more often prepared with the fluid method than with the smear method. Moreover, the use of separate needles for each technique eliminated this possible bias in the second half of the study.

TBNA via flexible bronchoscopy is a technically advanced, minimally invasive, cost-effective, and elegant sampling procedure. To achieve a regular high yield with TBNA, however, careful attention to technical detail is essential. Smear preparation of a TBNA sample can be easily learned and will increase the diagnostic yield of TBNA at very low cost.

References

1 Minai OA, Dasgupta A, Mehta AC. Transbronchial needle aspiration of central and peripheral lesions. In: Bolliger CT, Mathur PN, eds. Interventional bronchoscopy. Basel, Switzerland: Karger, 2000; 66–79

2 Clifton F, Mountain MD. Revisions in the international system for staging lung cancer. Chest 1997; 111:1711–1717

3 Mazzone P, Jain P, Arroliga AC, at al. Bronchoscopy and needle biopsy techniques for diagnosis and staging of lung cancer. Clin Chest Med 2002; 23:137–158

4 Dasgupta A, Jain P, Minai OA, et al. Utility of transbronchial needle aspiration in the diagnosis of endobronchial lesions. Chest 1999; 115:1237–1241

5 Dasgupta A, Mehta AC. Transbronchial needle aspiration: an underused diagnostic technique. Clin Chest Med 1999; 20: 39–51

6 Hsu LH, Liu CC, Ko JS. Education and experience improve the performance of transbronchial needle aspiration: a learning curve at a cancer center. Chest 2004; 125:532–540

7 Wang KP, Brower R, Haponik EF, et al. Flexible transbronchial needle aspiration for staging of bronchogenic carcinoma. Chest 1983; 84:571–576

8 Wang KP, Selcuk ZT, Erozan Y. Transbronchial needle aspiration for cytology specimens. Monaldi Arch Chest Dis 1994; 49:265–267

9 Rosenthal DL, Wallace JM. Fine needle aspiration of pulmonary lesions via fiberoptic bronchoscope. Acta Cytol 1982; 28:203–210

10 Wang KP. Staging of bronchogenic carcinoma by bronchoscopy. In: Wang KP, Metha AC, eds. Flexible bronchoscopy. Cambridge, UK: Blackwell Science, 1995; 6–17

11 Keebler KM. Cytopathology techniques. In: Bibbo M, ed. Comprehensive cytopathology. 2nd ed. Philadelphia PA: WB Saunders 1997; 881–906

12 Diacon AH, Schuurmans MM, Theron J, et al. Utility of rapid on-site evaluation of transbronchial needle aspirates. Respiration 2005; 72:182–188

13 Mehta AC, Kavuru MS, Meeker DP, et al. Transbronchial needle aspiration for histology specimens. Chest 1989; 96: 1228–1232

Appendix F

Transthoracic needle aspirates: how many passes per target site?

European Respiratory Journal 2007; 29: 112-116

Eur Respir J 2007; 29: 112–116
DOI: 10.1183/09031936.00055506

Transbronchial needle aspirates: how many passes per target site?

A.H. Diacon*, M.M. Schuurmans*, J. Theron*, K. Brundyn#, M. Louw#, C.A. Wright# and C.T. Bolliger*

ABSTRACT: Transbronchial needle aspiration is a bronchoscopic sampling method for a variety of bronchial and pulmonary lesions. The present study investigated whether and how serial needle passes contribute to the yield of transbronchial needle aspiration at specific target sites.

A total of 1,562 needle passes, performed at 374 target sites in 245 patients with neoplastic disease (82%), non-neoplastic disease (15%) or undiagnosed lesions (3%), were prospectively recorded and rated for anatomical location, size, bronchoscopic appearance and underlying disease.

Positive aspirates were obtained in 75% of patients and at 68% of target sites. A diagnosis was established with the first, second, third and fourth needle pass at 64, 87, 95 and 98% of targets, respectively. The absolute yield varied strongly with target site features, but the stepwise increment to the maximum yield provided by serial passes was similar across target sites.

In conclusion, three transbronchial needle passes per site are appropriate when only a tissue diagnosis is sought and when alternative sites or sampling modalities are available. At least four or five passes should be carried out at lymph node stations critical for the staging of lung cancer.

KEYWORDS: Bronchoscopy, cytodiagnosis, fine-needle biopsy, lung neoplasms, neoplasm staging

AFFILIATIONS
Depts of *Internal Medicine, and #Anatomical Pathology, Tygerberg Academic Hospital, University of Stellenbosch, Cape Town, South Africa.

CORRESPONDENCE
A.H. Diacon
Dept of Internal Medicine
PO Box 19063
7505 Tygerberg
South Africa
Fax: 27 219317442
E-mail: ahd@sun.ac.za

Received:
April 23 2006
Accepted after revision:
September 14 2006

SUPPORT STATEMENT
A.H. Diacon was supported by a grant from the University of Stellenbosch (Cape Town, South Africa). None of the authors has a financial interest to declare.

Transbronchial needle aspiration (TBNA) *via* flexible bronchoscopy (FB) is a well-established sampling method for a variety of bronchial, peribronchial or pulmonary lesions [1]. Its ability to establish diagnosis and staging in a single noninvasive intervention has made TBNA the key technique for the evaluation of patients with suspected lung cancer [2, 3]. Endobronchial ultrasound (EBUS) [4], computed tomography (CT) guidance [5] and rapid on-site evaluation (ROSE) improve TBNA yield [6, 7], but these methods require considerable resources and are not universally available. In the absence of EBUS and/or ROSE, it is common practice to perform several TBNA passes at a target site to minimise false-negative results. However, little is known about the value of serial aspirations. CHIN *et al*. [8] reported a plateau in yield after seven aspirates per patient and per nodal site, while other authors have reported the performance of two [9], two to three [10, 11], at least three [12], three to four [13] or three to five [14, 15] passes per site. It is well known that TBNA has a higher yield in neoplastic than in benign lesions, as well as in small cell lung cancer (SCLC) compared with nonsmall cell lung cancer (NSCLC) [3, 16]. Other predictors of positive aspirates are greater size of lymph nodes, infracarinal or right tracheobronchial position, visible mucosal abnormalities, such as a widened carina or erythema, and endobronchial mass lesions [1, 3, 9, 10, 17]. It is unknown whether these parameters also predict a higher yield when fewer aspirates are performed at these sites.

The demonstration of positive N2 or N3 lymph nodes using TBNA avoids unnecessary surgical exploration, with its associated morbidity and cost [3, 18]. Such procedures often require TBNA sampling of multiple sites, proceeding in a stepwise fashion from the highest-rated potentially involved nodal site to the primary tumour, followed by additional sampling modalities. Patient comfort and safety challenge the bronchoscopist to find an optimal compromise between TBNA yield and (possibly unnecessary) prolongation of the intervention. The present study investigated the yield of serial TBNA as a function of target site characteristics, with the aim of establishing a practical rule for sampling in routine practice.

METHODS

Patients, interventions and diagnoses

All patients undergoing FB with TBNA at the present authors' tertiary academic hospital (Tygerberg Academic Hospital, Cape Town,

European Respiratory Journal
Print ISSN 0903-1936
Online ISSN 1399-3003

South Africa) from June 2001 to June 2004 were prospectively recorded. Four chest physicians experienced in TBNA performed all procedures, using standard fibreoptic or video bronchoscopes (models BF30 and BF1T160; Olympus, Hamburg, Germany) and standard TBNA for cytological specimens (Bard, Billerica, MA, USA) under topical anaesthesia (lidocaine 1%) and conscious sedation (midazolam *i.v.* as needed). TBNA was always the leading sampling method and was supplemented at the discretion of the physician with appropriate additional modalities. For staging of suspected lung cancer, the potentially highest-rated nodal site was sampled first. If staging was not of concern, the most promising site for providing a diagnosis was sampled first. The final diagnosis was established using the results of the bronchoscopy or, in cases with negative FB, with appropriate repeat or additional examinations. All patients signed informed consent. The study was approved by the institutional ethical review board.

TBNA and target sites

A target site for TBNA was defined as an area of interest on CT (anatomical lymph node station or other lesion within reach of TBNA) or a visible abnormality identified during FB. Target site features were prospectively recorded. At least five successive aspirates in close proximity were performed. Every aspirate was immediately expressed onto a numbered glass slide and reported separately. TBNA sampling ended when all target sites had been aspirated or when sufficient diagnostic material was found with ROSE. ROSE was performed by a cytopathologist as previously described [7]. The anatomical location of lymph node target sites was classified according to the American Thoracic Society system [18] into paratracheal sites above the tracheobronchial level (stations 2R and 2L), tracheobronchial sites (stations 4R, 4L and 7) and bronchial sites (all sites below tracheobronchial). All sites were rated for normal or altered appearance (*i.e.* widened carina, mucosal infiltration, extrinsic compression). Compression of a lumen was rated for its degree as partial or complete (passable with bronchoscope or not) and for appearance (intrabronchial mass lesion opposed to submucosal or peribronchial disease). Post-bronchoscopy, the sites were further categorised for underlying disease (neoplastic or benign), type of lung cancer when applicable (SCLC or NSCLC), and short axis diameter in the case of tracheobronchial lymph nodes (assessed on contrasted spiral CT scan with 10-mm sections).

Statistical analysis

It was anticipated that sequential passes at a target site would result in stepwise yield increments to a plateau. Based on evidence in a published report [8], it was decided that five aspirates per site would provide sufficient data points to fit an exponential function with nonlinear regression (Newton–Gauss). Every needle pass at a site was reported separately and entered into a database to provide yields after each sequential pass. Using these data, separate exponential functions were created to deduct the yields stratified for target site characteristics. Proportional data were analysed using a Chi-squared test of contingency tables or Fisher's exact test on 2×2 contingency tables in the case of very small counts ($\leqslant 5$). A p-value <0.05 was considered significant. Two-sided tests were used. Data are presented as mean$\pm$SD unless otherwise stated.

RESULTS

Patients, diagnosis and interventions

A total of 245 patients undergoing flexible bronchoscopy with TBNA were recorded (age range: 15–88 yrs; median 57 yrs; 66% male). The final diagnosis was neoplastic disease in 200 (82%) and non-neoplastic disease in 36 (15%). Nine (3%) remained undiagnosed (table 1). Five of these patients died from clinically advanced malignancy before further investigations could be undertaken. One patient died undiagnosed from massive haemoptysis and three were lost to follow-up. TBNA was diagnostic in 75% of patients overall, in 84% with neoplastic disease and in 44% with benign lesions.

Target sites

In total, 374 target sites were sampled (1.53 ± 0.6 per patient) with 1,562 needle passes (4.2 ± 1.6 per site; range 1–10). The site-specific yields are demonstrated in table 2. More than half of all target sites were at the tracheobronchial level (stations 4R, 4L, 7). Significantly higher yields were seen with increasing size of lymph nodes and at the tracheobronchial level in right-sided (station 4R) and infracarinal (station 7) compared to left-sided (station 4L) lymph nodes. Other statistically significant predictors of positive aspirates were the presence of a visible abnormality and neoplastic disease. Among abnormally appearing sites, endobronchial mass lesions were significantly more often positive than submucosal or peribronchial lesions. Of borderline significance was the better yield in SCLC compared to NSCLC. There was no significant difference between partial and complete endobronchial obstruction.

Sequential yield of TBNA

The cumulative yield obtained from the complete set of 1,562 needle aspirates at 374 sites is displayed in figure 1. The first

TABLE 1 Bronchoscopies, diagnoses and yield of transbronchial needle aspiration (TBNA)

	Underlying disease	Diagnostic TBNA[#]
All	245 (100)	75
Neoplastic disease	200 (82)	84
Nonsmall cell lung cancer	154 (63)	81
Adenocarcinoma	82 (34)	85
Squamous cell carcinoma	25 (10)	84
Undifferentiated carcinoma	47 (19)	72
Small cell lung cancer	39 (16)	95
Other neoplastic[¶]	7 (3)	86
Non-neoplastic disease	36 (15)	44
Sarcoidosis	10 (4)	60
Tuberculosis	14 (6)	71
Other infective lesions	5 (2)	0
Other benign lesions	7 (3)	0
Undiagnosed	9 (3)	0

Data are presented as n (%) or %. [#]: at least one diagnostic aspirate; [¶]: metastasis (n=4), lymphoma (n=2), myeloma (n=1).

TABLE 2 Target sites and yield of transbronchial needle aspiration

	Target sites aspirated	Diagnostic#	p-value¶	
All sites	374 (100)	68		
Anatomical location+				
Paratracheal sites§	32 (9)	75	0.043	NS
Tracheobronchial sites‡	212 (57)	56	<0.001	
Bronchial sites	130 (35)	85		
Underlying disease				
Neoplastic	309 (83)	76	<0.001	
Non-neoplastic	55 (15)	35		
Bronchial carcinoma				
Small cell lung cancer	64 (17)	86	0.052	
Nonsmall cell lung cancer	230 (61)	74		
Paratracheal and tracheobronchial lymph node sites				
Visible abnormality				
Present	124 (33)	75	<0.001	
Absent	120 (32)	42		
Lymph node short axis diameter mm				
⩽10	39 (10)	28	0.004	<0.001
11–20	97 (26)	56	0.016	
>20	108 (29)	72		
Tracheobronchial position				
Right##	49 (13)	61	0.004	NS
Left¶¶	34 (9)	29	<0.001	
Subcarinal++	129 (34)	61		
Bronchial sites				
Degree of bronchial obstruction				
None or partial	70 (19)	80	NS	
Complete	60 (16)	92		
Bronchoscopic appearance				
Peribronchial or submucosal disease	76 (20)	76	<0.001	
Endobronchial mass lesion	54 (14)	98		

Data are presented as n (%) and %, unless otherwise stated. NS: nonsignificant. #: at least one diagnostic transbronchial needle aspiration per target site. ¶: Where the Diagnostic column shows three values consecutively, the figures in left-hand part of the p-value column show a comparison of the first and second and then the second and third values; values in the right-hand p-value column show a comparison of the first and third values in the Diagnostic column. +: lymph node station according to the American Thoracic Society system [18]. §: stations 2R, 2L. ‡: stations 4R, 4L, 7. ##: station 4R. ¶¶: station 4L. ++: station 7.

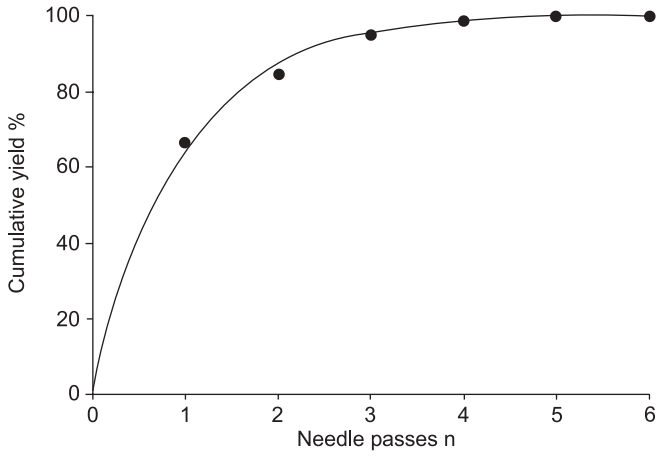

FIGURE 1. Incremental yield to plateau with sequential needle passes. The graph describes the yield to plateau in 1,562 transbronchial needle aspiration passes at 374 target sites. ●: measured yield after each sequential needle pass; ——: extrapolated yield from an exponential function, obtained by nonlinear regression. The function is "yield=100-b0 × $e^{(b1 \times \text{needle passes})}$", where the coefficients b0 and b1 determine the shape of the curve. The correlation is excellent (R^2=0.999).

needle pass contributed the largest proportion to the total yield at all sites, and all following passes increased the yield by ~50% of the increase brought about by the previous pass until a plateau was reached. As expected, this pattern could be described with a simple nonlinear function. The functions and graphs established for sites with specific features were very similar to figure 1 (data not shown; all correlation coefficients R^2>0.96). Table 3 shows the proportional yields of the plateau yield after the first five sequential passes for each site. The highest first-pass contribution was achieved at sites with complete airway obstruction (82.6%) and in endobronchial mass lesions (76.7%). At all sites, ⩾88% of the plateau yield was reached with three passes and ⩾94% with four needle passes.

DISCUSSION

The present study showed that the stepwise increase in TBNA yield with serial needle passes is similar across target sites of variable position, aspect, size and underlying disease. Although the rate of positive TBNA was significantly different across target sites, the first needle pass consistently contributed ⩾50% towards the maximum yield, three passes provided 89–99%, and five passes yielded ⩾98% at all sites. TBNA was

TABLE 3 Target sites and yield of sequential needle passes

	Yield after needle pass					Fit[#] R^2
	1	2	3	4	5	
All sites	64.5	87.4	95.5	98.4	99.4	0.998
Anatomical location[¶]						
Paratracheal sites[+]	59.6	83.8	93.5	97.4	99.0	0.996
Tracheobronchial sites[§]	57.4	82.0	92.4	96.8	98.8	0.997
Bronchial sites	73.5	93.0	98.1	99.5	99.9	0.996
Underlying disease						
Neoplastic	64.2	87.2	95.4	98.3	99.4	0.998
Non-neoplastic	68	89.7	96.7	98.9	99.7	0.978
Bronchial carcinoma						
Small cell lung cancer	59.5	83.6	93.4	97.3	98.9	0.998
Nonsmall cell lung cancer	65.8	88.3	96.0	98.6	99.5	0.998
Paratracheal and tracheobronchial lymph node sites						
Visible abnormality						
Present	53.4	78.2	89.8	95.2	97.8	0.991
Absent	60.1	84.2	93.7	97.5	99.0	0.998
Lymph node short axis diameter mm						
⩽10	64.8	87.8	95.8	98.5	99.5	0.968
11–20	59.2	83.2	93.1	97.2	98.8	0.995
>20	51.5	76.7	88.8	94.6	98.8	0.991
Tracheobronchial position						
Right[ƒ]	54.5	79.4	90.7	95.8	98.1	0.995
Left[##]	60.3	84.3	93.8	97.5	99.0	0.992
Subcarinal[¶¶]	57.1	81.7	92.2	96.7	98.6	0.998
Bronchial sites						
Degree of bronchial obstruction						
None or partial	67.4	89.3	96.5	98.9	99.6	0.997
Complete	82.6	97.0	99.5	99.9	100.0	0.992
Bronchoscopic appearance						
Peribronchial or submucosal disease	71.2	91.7	97.6	99.3	99.8	0.996
Endobronchial mass lesion	76.7	94.6	98.7	99.7	99.9	0.996

Data presented as %, unless otherwise stated. [#]: nonlinear function curve fit (see fig. 1); [¶]: lymph node station according to the American Thoracic Society system [18]; [+]: stations 2R, 2L; [§]: stations 4R, 4L, 7; [ƒ]: station 4R; [##]: station 4L; [¶¶]: station 7.

diagnostic in 75% of a large sample of patients representative for clinical practice.

The ideal number of TBNA passes per target site has not received much investigative attention in the past. One reason might be that a negative TBNA result can have a variety of causes, such as inadequate puncture technique or suboptimal sample preparation and analysis [17]. Secondly, TBNA has a sensitivity of only 76–80% in the best hands under study conditions [10, 19, 20], which means that a negative result is of limited value even when established with a high number of aspirates. ROSE by a cytopathologist present in theatre will effectively optimise the number of aspirates in patients with positive TBNA but will contribute little when TBNA remains negative [7, 21]. In contrast, EBUS improves TBNA sensitivity by assisting the positioning of the needle inside the target lesion [4]. However, the majority of chest physicians performing TBNA do not have easy access to EBUS or ROSE and will rely on their clinical judgement and personal experience to decide on the number of aspirates in specific bronchoscopic situations.

Tracheobronchial lymph-node sampling for staging of lung cancer is the best established and most widely used indication for TBNA. The current results in this subgroup of sites confirm previous reports that the yield of TBNA is strongly influenced by the size and location of the targeted lymph node, as well as by the presence of erythema and a widened carina [3, 16]. Even though radiological size is a poor predictor for disease in the mediastinum [22], the current authors' yield in small nodes (<10 mm small axis diameter: 29% yield) is surprisingly high. HARROW *et al.* [10] reported a TBNA yield in tracheobronchial lymph nodes <10 mm of 14% in a large sample of patients with lung cancer. The explanation for this discrepancy may be the inclusion of nodes measuring exactly 10 mm into that group in the present study. The current authors' yield in nodes measuring <10 mm was only 16%. For sites other than tracheobronchial, the present study confirms the prediction of positive aspirates by visible abnormalities such as a widened carina, submucosal infiltration, airway compression or endobronchial mass lesions [9, 16, 17].

The good overall yield of 75% in the present study encourages the use of TBNA regardless of the availability of EBUS support. While EBUS-guided TBNA is superior in lymph-node targets <10 mm [23] or in peripheral lung lesions [24], most parabronchial lesions can be located using anatomical landmarks, such as the carina or lobar bifurcations [25, 26]. Moreover, positive ROSE-TBNA makes EBUS redundant and shortens the sampling process [7]. The preferred method for mediastinal staging will not only depend on the available expertise but also on the prevalence of mediastinal metastases. HOLTY *et al.* [20] have recently shown that TBNA has a higher sensitivity in more advanced mediastinal disease than in situations with small lymph nodes. This means that non-EBUS-TBNA is probably sufficient for the majority of patients for whom confirmation of inoperability is sought. Conversely, EBUS-TBNA or even surgical staging is best used when a surgically operable stage is suspected and a high negative predictive value is important.

In conclusion, what can be recommended for general practice? Transbronchial needle aspiration is an elegant and effective technique that takes bronchoscopic sampling beyond visible abnormalities. Even though other sampling methods are often equally promising, frequent practice of transbronchial needle aspiration to hone technical skills is to be encouraged. In general, it seems reasonable to perform three serial transbronchial needle aspiration passes per site when the main objective is establishing a tissue diagnosis and when alternative target sites or other sampling modalities are equally promising. Four or even five transbronchial needle aspiration passes per site should be carried out if only a single site is available, if transbronchial needle aspiration is the only potentially diagnostic sampling method, and if the objective is staging of lung cancer at critical lymph node stations.

REFERENCES

1 Mazzone P, Jain P, Arroliga AC, Matthay RA. Bronchoscopy and needle biopsy techniques for diagnosis and staging of lung cancer. *Clin Chest Med* 2002; 23: 137–158.
2 Gasparini S, Silvestri GA. Usefulness of transbronchial needle aspiration in evaluating patients with lung cancer. *Thorax* 2005; 60: 890–891.
3 Minai OA, Dasgupta A, Mehta AC. Transbronchial needle aspiration of central and peripheral lesions. *In*: Bolliger CT, Mathur PN, eds. Interventional Bronchoscopy. Basel, Karger, 2000; pp. 66–79.
4 Herth FJ, Becker HD, Ernst A. Ultrasound-guided transbronchial needle aspiration: an experience in 242 patients. *Chest* 2003; 123: 604–607.
5 Geraghty PR, Kee ST, McFarlane G, Razavi MK, Sze DY, Dake MD. CT-guided transthoracic needle aspiration biopsy of pulmonary nodules: needle size and pneumothorax rate. *Radiology* 2003; 229: 475–481.
6 Davenport RD. Rapid on-site evaluation of transbronchial aspirates. *Chest* 1990; 98: 59–61.
7 Diacon AH, Schuurmans MM, Theron J, *et al.* Utility of rapid on-site evaluation of transbronchial needle aspirates. *Respiration* 2005; 72: 182–188.
8 Chin R, McCain TW, Lucia MA, *et al.* Transbronchial needle aspiration in diagnosing and staging lung cancer: how many aspirates are needed? *Am J Respir Crit Care Med* 2002; 166: 377–381.
9 Harrow E, Halber M, Hardy S, Halteman W. Bronchoscopic and roentgenographic correlates of a positive transbronchial needle aspiration in the staging of lung cancer. *Chest* 1991; 100: 1592–1596.
10 Harrow EM, Abi-Saleh W, Blum J, *et al.* The utility of transbronchial needle aspiration in the staging of bronchogenic carcinoma. *Am J Respir Crit Care Med* 2000; 161: 601–607.
11 Wang KP, Brower R, Haponik EF, Siegelman S. Flexible transbronchial needle aspiration for staging of bronchogenic carcinoma. *Chest* 1983; 84: 571–576.
12 Shure D. Transbronchial biopsy and needle aspiration. *Chest* 1989; 95: 1130–1138.
13 Schenk DA, Chambers SL, Derdak S, *et al.* Comparison of the Wang 19-gauge and 22-gauge needles in the mediastinal staging of lung cancer. *Am Rev Respir Dis* 1993; 147: 1251–1258.
14 Schenk DA, Bower JH, Bryan CL, *et al.* Transbronchial needle aspiration staging of bronchial carcinoma. *Am Rev Respir Dis* 1986; 134: 146–148.
15 Schenk DA, Bryan CL, Bower JH, Myers DL. Transbronchial needle aspiration in the diagosis of bronchogenic carcinoma. *Chest* 1987; 92: 83–85.
16 Utz JP, Patel AM, Edell ES. The role of transcarinal needle aspiration in the staging of bronchogenic carcinoma. *Chest* 1993; 104: 1012–1016.
17 Haponik EF, Cappellari JO, Chin R, *et al.* Education and experience improve transbronchial needle aspiration performance. *Am J Respir Crit Care Med* 1995; 151: 1998–2002.
18 Mountain CF, Dresler CM. Regional lymph node classification for lung cancer staging. *Chest* 1997; 111: 1718–1723.
19 Toloza EM, Harpole L, Detterbeck F, McCrory DC. Invasive staging of non-small cell lung cancer: a review of the current evidence. *Chest* 2003; 123: Suppl. 1, 157S–166S.
20 Holty JE, Kuschner WG, Gould MK. Accuracy of transbronchial needle aspiration for mediastinal staging of non-small cell lung cancer: a meta-analysis. *Thorax* 2005; 60: 949–955.
21 Baram D, Garcia RB, Richman PS. Impact of rapid on-site cytologic evaluation during transbronchial needle aspiration. *Chest* 2005; 128: 869–875.
22 Toloza EM, Harpole L, McCrory DC. Noninvasive staging of non-small cell lung cancer: a review of the current evidence. *Chest* 2003; 123: Suppl. 1, 137S–146S.
23 Herth FJ, Ernst A, Eberhardt R, Vilmann P, Dienemann H, Krasnik M. Endobronchial ultrasound-guided transbronchial needle aspiration of lymph nodes in the radiologically normal mediastinum. *Eur Respir J* 2006; 28: 910–914.
24 Paone G, Nicastri E, Lucantoni G, *et al.* Endobronchial ultrasound-driven biopsy in the diagnosis of peripheral lung lesions. *Chest* 2005; 128: 3551–3557.
25 Herth F, Becker HD, Ernst A. Conventional *vs* endobronchial ultrasound-guided transbronchial needle aspiration: a randomized trial. *Chest* 2004; 125: 322–325.
26 Trisolini R, Agli LL, Patelli M. Conventional *vs* endobronchial ultrasound-guided transbronchial needle aspiration of the mediastinum. *Chest* 2004; 126: 1005–1006.

Appendix G

Ultrasound assisted transthoracic biopsy: fine needle aspiration or cutting needle biopsy?

European Respiratory Journal 2007; 29: 357-362

Eur Respir J 2007; 29: 357–362
DOI: 10.1183/09031936.00077706

Ultrasound-assisted transthoracic biopsy: fine-needle aspiration or cutting-needle biopsy?

A.H. Diacon*, J. Theron*, P. Schubert#, K. Brundyn#, M. Louw#, C.A. Wright# and C.T. Bolliger*

ABSTRACT: The present study compared the diagnostic yield of ultrasound-assisted cutting-needle biopsy (CNB) and fine-needle aspiration biopsy (FNAB) in chest lesions.

A physician performed ultrasound and FNAB with a 22-G spinal needle in all patients, directly followed by a 14-G CNB in patients without contraindication.

A total of 155 consecutive lesions arising from the lung (74%), pleura (12%), mediastinum (11%) or chest wall (3%) in patients with a final diagnosis of lung carcinoma (74%), other malignant tumours (12%), non-neoplastic disease (9%) or unknown (5%) were prospectively included. The overall diagnostic yield was 87%. Combined specimens were obtained in 123 lesions (79%). In these, yields of FNAB, CNB and both methods combined were 82, 76 and 89%, respectively. FNAB was significantly better than CNB in lung carcinoma (95 *versus* 81%) but CNB was superior in noncarcinomatous tumours and in benign lesions. On-site cytology was 90% sensitive and 100% specific for predicting a positive FNAB. One patient required drainage for pneumothorax (0.6%).

Ultrasound-assisted fine-needle aspiration biopsy performed by chest physicians is an accurate and safe initial diagnostic procedure in patients with a high clinical probability of lung carcinoma. All other patients should undergo concurrent fine-needle aspiration biopsy and cutting-needle biopsy.

KEYWORDS: Cutting-needle biopsy, fine-needle aspiration, lung biopsy, lung carcinoma, pleural biopsy, ultrasound

Ultrasound (US) has found a firm place in chest medicine as an aid for assessing pleural effusions at the bedside [1]. This development was facilitated by the advent of affordable, lightweight and mobile US units. Although less practised by physicians, US can also visualise solid lesions arising from the pleura, chest wall and anterior mediastinum, and even lung tumours and consolidations are detected without difficulty provided they extend to the parietal pleura. US is an ideal tool to assist with biopsy procedures. It can frequently replace computed tomographic (CT) guidance at much lower cost [2].

US-assisted cutting-needle biopsy (CNB) performed by chest physicians was 100% sensitive for mesothelioma in a recent prospective study carried out at the present authors' institution [3]. In that study, the majority of candidates for US-assisted biopsy did not suffer from pleural disease but presented with peripheral lung carcinoma, for which fine-needle aspiration biopsy (FNAB) might be a safer and technically easier alternative to CNB. US-assisted FNAB integrates easily with routine practice, as it only requires basic ultrasound equipment and consumables commonly used by chest physicians for diagnostic thoracentesis or lymph node aspiration. FNAB and CNB both have a high diagnostic yield for lung carcinoma under CT guidance [4, 5], which is essential for lung lesions located within the lung parenchyma. In contrast, US can only be used on lesions with pleural contact, which frequently represent rather large and partly necrotic lung tumours, inflammatory processes or consolidations due to central bronchial obstruction. It is unknown which biopsy device performs better in chest lesions accessible to US. The present study directly compared the diagnostic yield of US-assisted transthoracic FNAB and CNB.

PATIENTS AND METHODS

All patients referred to the Lung Unit of Tygerberg Hospital (Cape Town, South Africa;

AFFILIATIONS
*Depts of Internal Medicine and #Anatomical Pathology, Tygerberg Academic Hospital and National Health Laboratory Service, University of Stellenbosch, Cape Town, South Africa.

CORRESPONDENCE
A.H. Diacon
Dept of Internal Medicine
PO Box 19063
7505 Tygerberg
South Africa
Fax: 27 219317442
E-mail: ahd@sun.ac.za

Received:
June 14 2006
Accepted after revision:
October 22 2006

SUPPORT STATEMENT
A.H. Diacon was supported by a grant of the University of Stellenbosch. The authors thank the Holland Stellenbosch Medical Foundation, Veldhoven, The Netherlands, for their continued support.

STATEMENT OF INTEREST
None declared.

European Respiratory Journal
Print ISSN 0903-1936
Online ISSN 1399-3003

tertiary academic centre, catchment area 1.5 million) with lesions on chest radiography or CT suspected to involve the chest wall or the pleura were candidates for the present study. Patients with pleural effusions were not enrolled but were further evaluated with pleural fluid cytology or thoracoscopy. A consultant respiratory physician or a registrar under supervision performed sonography (Toshiba Sonolayer L SAL 77A, 3.75-MHz sector probe; Toshiba, Johannesburg, South Africa) and biopsy in free-hand technique (no dedicated biopsy sonar probe) in a bronchoscopy suite. All patients gave signed informed consent. Ethical approval from the institutional review board was obtained.

Biopsy procedure

After consulting the radiological evidence the target lesion was identified with US and the most suitable patient position, as well as entry site, direction and depth for the biopsy, were determined. Under local anaesthesia with lidocaine 1%, the lesion was first aspirated with a 22-G spinal needle (38 or 90 mm; Becton Dickinson, Madrid, Spain) connected to a 10-mL syringe. Three passes from slightly different areas of the target were directly expressed onto slides and smeared. The slides were either air dried or submitted for rapid on-site analysis (ROSE) using Diff-Quik (Rapidiff; Clinical Sciences Diagnostics, Southdale, South Africa) or rapid Papanicolaou staining methods [6]. Immediately thereafter, two or more CNB passes were performed at the same location until macroscopically satisfactory material was harvested (14-G cutting needle, manually driven, specimen notch 20 mm; Allegiance, Chateaubriand, France). To ensure a consistent effort with the CNB procedure, the operator was only informed about the ROSE result upon completion of the CNB. When the lesions were small (<3 cm in diameter), mobile, in close vicinity to vital structures, or when a patient was in poor medical condition, it was left to the physician to decide whether or not to perform a CNB. The reason for not performing CNB was documented by the physician. Directly after the procedure, the site was re-examined with US. A follow-up chest radiograph was obtained if the pre- and post-biopsy US findings differed and at the discretion of the physician.

Assessments and measurements

Lesions were classified as follows on radiological appearance as to the most likely origin: ''pleural'' (pleural based, blunt angle to the lung), ''pulmonary'' (lesion with centre in the lung, acute angle to the pleura), ''chest wall'' (lesions centred in the chest wall with pleural involvement) or ''mediastinal'' (lesions predominantly located in the anterior mediastinum with extension to the pleura). The maximum depth of the target lesion was measured sonographically in the direction of the planned puncture. If the target extended deeper than 100 mm from the skin, 100 mm was noted. As an indication for locally advanced disease, the presence of superior vena cava syndrome or signs of direct spread into nerval tissue (nerve roots, spinal cord, brachial plexus) was recorded. This would indicate surgical nonresectability in the setting of nonsmall cell lung carcinoma.

Scoring and statistical analysis

Specimens with an unequivocal diagnosis of neoplastic disease or a positive stain or culture for mycobacteria were accepted as diagnostic. Nondiagnostic specimens contained normal cells or tissue, nonrepresentative material, or necrotic cells or tissue. Typing discrepancies between nonsmall cell lung cancer (NSCLC) subgroups were not considered typing errors because dual differentiation is common and it is not of therapeutic relevance. The accuracy of ROSE for predicting diagnostic material on-site was established by comparison with the final FNAB result. Patients with nondiagnostic FNAB and CNB were either clinically observed or further investigated by the appropriate means. Two different pathologists independently reviewed FNAB and CNB specimens. Descriptive statistics and Chi-squared comparisons of proportional data were performed. A p-value <0.05 in a two-tailed test of proportions (Chi-squared) was considered significant.

RESULTS

Patients and lesions

A total of 155 patients were included over a 23-month period. Median age was 56 yrs (range 27–90 yrs), 68% were male and 50% were outpatients. The majority of cases were lung lesions extending to the pleura (n=115, 74%), followed by pleural-based lesions (n=18, 12%), mediastinal lesions (n=17, 11%) and lesions situated in the chest wall (n=5, 3%). At the time of biopsy, a chest radiograph was available in all patients and a chest CT scan in 100 patients (64%). Locally advanced disease with clinical signs of superior vena cava compression was noted in 20 patients (13%) and invasion of spinal or plexus nerval tissue in 12 (8%). Mean maximum lesion depth from the skin was 73 mm (range 20–100 mm).

TABLE 1 Diagnosis and yield of fine-needle aspiration biopsy (FNAB) and cutting-needle biopsy (CNB)

	Final diagnosis	Diagnostic FNAB and/or CNB
All	155 (100)	135 (87)
Malignant lesions	133 (86)	126 (95)
Lung carcinoma	114 (74)	110 (96)
Small cell carcinoma	11 (7)	11 (100)
Adenocarcinoma	37 (24)	37 (100)
Squamous cell carcinoma	16 (10)	16 (100)
Undifferentiated carcinoma	50 (32)	46 (92)
Sarcoma	8 (5)	8 (100)
Lymphoma	5 (3)	2 (40)
Mesothelioma	2 (1)	2 (100)
Other malignant[#]	4 (3)	4 (100)
Benign lesions	14 (9)	9 (64)
Fibrosis	6 (4)	2 (33)
Tuberculosis	6 (4)	5 (83)
Lipoma	1 (1)	1 (100)
Wegener's granulomatosis	1 (1)	1 (100)
Undiagnosed	8 (5)	

Data are presented as n (%). [#]: Thymoma, carcinoid tumour, carcinoma with unknown primary, prostate cancer.

TABLE 2 Cases with negative biopsy procedures

Description	Cases n
Diagnosis established with other means	12
Fibrosis, confirmed with follow-up and full clinical recovery	4
Lymphoma, surgical biopsy	3
Undifferentiated nonsmall cell lung carcinoma, diagnosed with transbronchial needle aspiration	2
Undifferentiated nonsmall cell lung carcinoma, diagnosed with repeat FNAB/CNB	2
Full recovery on antituberculous treatment	1
Undiagnosed	8
Deceased from widely metastatic tumour from unknown primary. Patients wanted no further diagnostic procedures	5
Deceased with metastatic adenocarcinoma of the colon	1
Patient died from HIV-related illness. Aspirated material showed giant cells and granuloma. Culture for *Mycobacterium tuberculosis* remained negative	1
Large paravertebral mass. Multiple biopsies revealed necrotic material. Lost to follow-up	1

FNAB: fine-needle aspiration biopsy; CNB: cutting-needle biopsy.

Diagnosis, procedures and safety

A final diagnosis was established in 147 patients (95%). Most diagnoses were established with US-assisted CNB and/or FNAB (135 patients, 87%; table 1). Twelve patients (8%) were diagnosed by other means and eight patients (5%) remained undiagnosed (table 2). The physician decided not to perform CNB in 32 patients (21%) because of technical or patient-related reasons (table 3). The diagnostic yield in these patients was 81% (malignant lesions 92%; benign lesions 57%). Adverse events did not occur in patients undergoing FNAB only, and were infrequent and mostly minor in those with combined procedures. Pneumothorax was observed in two patients (1.3%), and in one of these, a chest drain was required (0.6%). Mild puncture-site bleeding (n=4, 2.6%), post-procedural pain requiring medication (n=2, 1.3%), vagovasal reaction (n=2, 1.3%) and transient mild haemoptysis (n=1, 0.6%) were minor events.

Histological diagnosis and correlation in paired samples

Paired diagnostic specimens (FNAB and CNB) were obtained in 123 patients (79%) with a combined diagnostic yield of 89% (table 4). The yield of FNAB and CNB alone was 82 and 76%, respectively (difference not significant). FNAB had a significantly higher yield than CNB in neoplastic disease (91 *versus* 82%, p=0.05), mainly due to a higher yield in lung carcinoma (95 *versus* 81%, p=0.006). Only one case of lung carcinoma (1%) was identified exclusively by CNB, whereas 14 cases (15%) were diagnosed with FNAB but not with CNB. Most of these tumours were of larger size than average and all showed necrosis on histology and/or necrotic areas on CT. Cytology and histology were concordant for SCLC and NSCLC in 74 out of 75 cases (99%). One case diagnosed as adenocarcinoma on cytology was classified as an atypical carcinoid on histology with immunohistochemistry, and this was later confirmed on resection of the tumour. This change in final diagnosis did not affect the management in that particular patient as surgical resection was the therapy of choice for both tumour types. When a diagnosis other than lung carcinoma was established (n=22), CNB was superior to FNAB with 18 *versus* 12 positive cases (82 *versus* 55%, p=0.056). However, in eight out of 12 cases with positive FNAB the definite classification was only possible with the additional information

TABLE 3 Reasons for not performing cutting needle biopsy in 32 patients

Description	Patients n
Lesion <30 mm and/or mobile beyond puncture site	11
Poor general medical condition with impaired cooperation	10
Lesion too close to vital structures	5
Abundant subcutaneous vascularisation in SVC obstruction syndrome	2
Inability to pass biopsy device between ribs	2
FNAB harvested fluid sample only, not suitable for biopsy	2

SVC: superior vena cava; FNAB: fine-needle aspiration biopsy.

TABLE 4 Diagnostic yield of fine-needle aspiration biopsy (FNAB) and cutting-needle biopsy (CNB) in paired samples

	Procedures	FNAB and CNB	FNAB alone	CNB alone	Chi-squared#
Total	123	109 (89)	101 (82)	94 (76)	NS
Neoplastic lesions	109	104 (95)	99 (91)	89 (82)	0.050
Lung carcinoma	94	91 (97)	89 (95)	76 (81)	0.006
Small cell lung carcinoma	8	8 (100)	7 (88)	7 (88)	NS
Nonsmall cell lung carcinoma	86	83 (97)	82 (95)	69 (80)	0.004
Neoplastic other than lung carcinoma	15	13 (87)	10 (67)	13 (87)	NS
Sarcoma	6	6 (100)	4 (67)	6 (100)	ND
Lymphoma	4	2 (50)	2 (50)	2 (50)	ND
Mesothelioma	2	2 (100)	1 (50)	2 (100)	ND
Other¶	3	3 (100)	3 (100)	3 (100)	ND
Benign lesions	7	5 (71)	2 (29)	5 (71)	NS
Fibrosis	4	2 (50)	0 (0)	2 (50)	ND
Tuberculosis, lipoma	2	2 (100)	2 (100)	2 (100)	ND
Wegener's granulomatosis	1	1 (100)	0 (0)	1 (100)	ND
All other than lung carcinoma	22	18 (82)	12 (55)	18 (82)	0.056
Undiagnosed	7				

Data are present as n and n (%), unless otherwise stated. ND: not done. NS: nonsignificant. #: Chi-squared, FNAB *versus* CNB; p-values $\leqslant 0.1$ are displayed, others are NS; ¶: Thymoma, carcinoma with unknown primary, carcinoid tumour (diagnosed as adenocarcinoma on FNAB).

from the CNB specimen (sarcoma n=4; lymphoma n=2; mesothelioma n=1; thymoma n=1). ROSE was 90% sensitive and 100% specific for predicting the presence of diagnostic material in positive FNAB.

DISCUSSION

The main finding of this prospective comparison of US-assisted FNAB and CNB in 155 chest lesions of mixed origin is the high combined diagnostic yield (87%), with a low rate of complications. FNAB and CNB are complementary techniques. Out of all the malignant tumours, 18% were diagnosed with only one modality. The yield of FNAB alone was significantly higher in lung carcinoma, while CNB was superior in all other malignant tumours or in benign diseases. The high yield (95%) of US-assisted FNAB in 89 cases of lung carcinoma is clinically relevant. Chest pain indicating pleural involvement is a common presenting symptom of lung caranoma, and a considerable proportion of these patients might qualify for US-assisted FNAB. This procedure is safe, can be performed at the bedside by a chest physician using the same tools as for a pleural tap or a diagnostic lymph node aspiration, and might be preferable to the more expensive and less convenient CT-guided approach in many cases.

Compared with the current authors' previous series of 91 US-assisted CNB [3], the high diagnostic yield has been maintained with a reduced pneumothorax rate. This is most probably due to the fact that FNAB replaced CNB in situations deemed by the investigator to carry an elevated risk for pneumothorax. It is reasonable to assume that FNAB causes less tissue damage than CNB and is therefore an inherently safer procedure. Small lesions, particularly when mobile on respiration (fig. 1), are at risk of being punctured slightly off centre. The chance of causing pneumothorax by accidental puncture of aerated tissue increases with the size of the biopsy device. Its safety, combined with its superior yield in lung carcinoma, makes US-assisted FNAB the first choice for biopsy of lesions originating from the lung. In contrast, lesions arising from the chest wall, pleura or mediastinum are, by their nature, safe targets for CNB because they are not mobile on respiration and are not surrounded by lung tissue. Such lesions are also less likely to represent carcinomatous tumours, which favour CNB due to the better yield in comparison to FNAB. As an added advantage, FNAB specimens are suited for ROSE, which is of proven value in transbronchial needle aspiration [7, 8], as well as in CT-guided aspiration of chest lesions [9, 10]. The sensitivity of ROSE for the presence of diagnostic material was 90% in the present study.

The superior yield of FNAB in lung carcinoma in the present study is intriguing. FNAB did not have a better yield than CNB in lung carcinomas in the large series by GONG *et al.* [4] reporting on CT-guided biopsies. This may partly be due to selection bias, because GONG *et al.* [4] only performed CNB when FNAB smears were considered suboptimal on immediate on-site assessment. Another explanation for the high rate of false-negative CNB could lie in the nature of neoplastic lung lesions detectable on US. Such lesions often show a non-homogenous picture with a mixture of necrotic areas, vital tumour and atelectatic lung tissue. A CNB pass can harvest a sizeable specimen from one area of the lesion, but this might only contain nonrepresentative material [5]. Moreover, scarce vital tumour tissue might easily be missed because only a small portion of a histological sample can routinely be cut and analysed under the microscope. In contrast, a fine needle passed through a lesion repeatedly can sample a

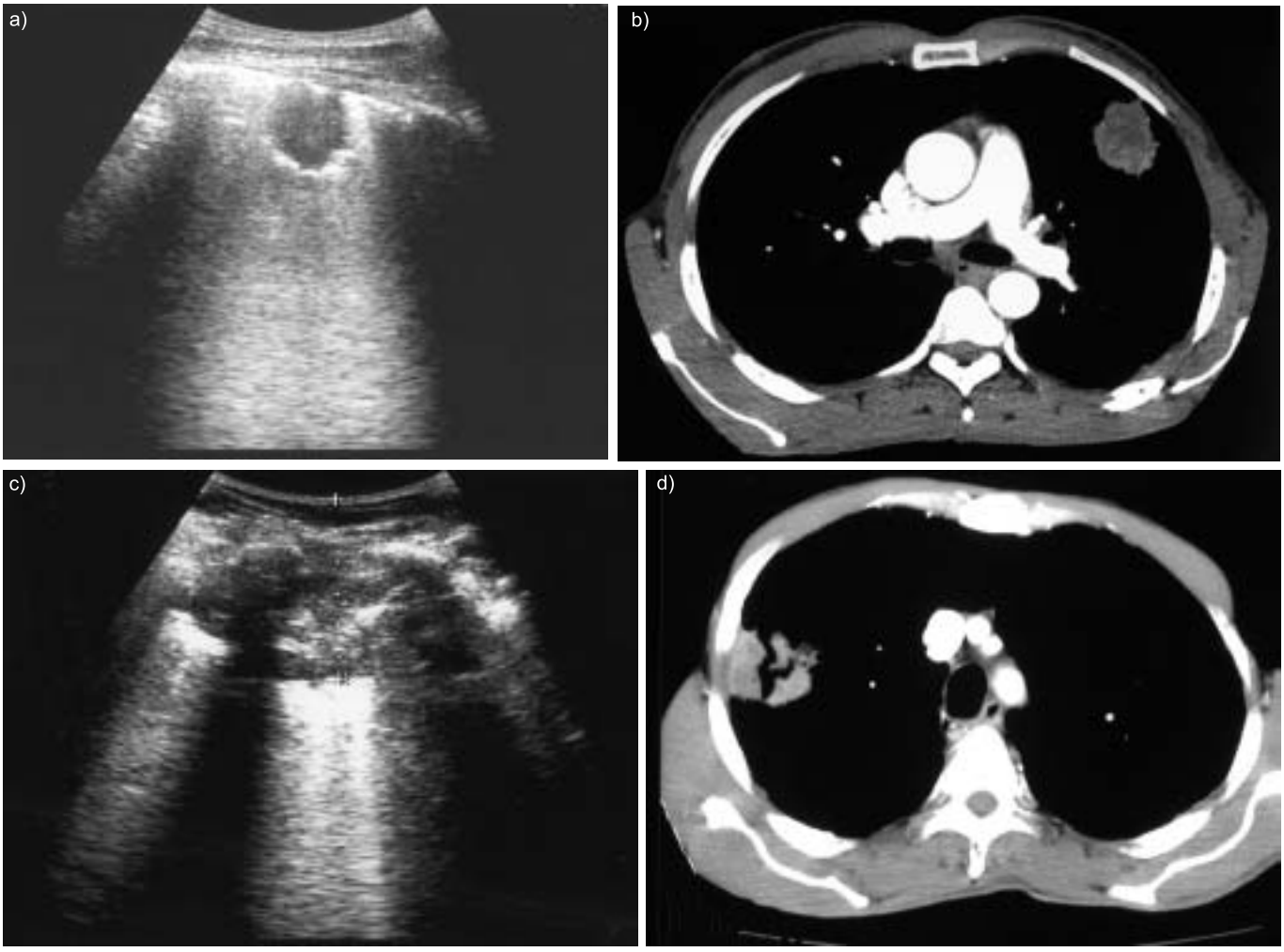

FIGURE 1. a) Sonographic image and b) computed tomography (CT) of a subpleural pulmonary tumour (40 × 30 mm on CT) that showed marked movement on respiration. The small area of contact with the pleura made its location with ultrasound (US) difficult. This patient only underwent fine-needle aspiration biopsy (FNAB). The diagnosis was adenocarcinoma. c) Sonographic image and d) CT of a lesion of similar size (40 × 30 mm on CT), arising from the lung and adherent to the pleura. The lesion showed no movement on respiration and the area of contact with the pleura was comparatively large. This lesion was easy to locate with US. This patient underwent FNAB and cutting biopsy without complications. The diagnosis was large cell lung carcinoma on both specimens.

comparatively large area. The specimen is less voluminous and can be completely scrutinised after direct transfer onto slides. This makes a positive result possible even when few vital cells have been collected. Tissue quality could also explain why CNB has a higher yield in nonepithelial malignant neoplasms. These tend to be homogenous and their high intercellular adherence makes aspiration difficult [4]. CNB specimens also facilitate the assessment of histological architecture and the performance of ancillary studies, which is often essential for classifying such tumours [4].

Despite similarly high yields, ultrasound-assisted fine-needle aspiration biopsy and cutting-needle biopsy are complementary methods for establishing a tissue diagnosis in patients with chest tumours involving the pleura. Considering its low degree of invasiveness, its simplicity, safety and low cost, ultrasound-assisted fine-needle aspiration biopsy is the method of choice in all patients with a high clinical probability of lung carcinoma. All other patients should undergo concurrent ultrasound-assisted fine-needle aspiration biopsy and cutting-needle biopsy.

REFERENCES

1 Beckh S, Bolcskei PL, Lessnau KD. Real-time chest ultrasonography: a comprehensive review for the pulmonologist. *Chest* 2002; 122: 1759–1773.
2 Diacon AH, Theron J, Bolliger CT. Transthoracic ultrasound for the pulmonologist. *Curr Opin Pulm Med* 2005; 11: 307–312.
3 Diacon AH, Schuurmans MM, Theron J, Schubert PT, Wright CA, Bolliger CT. Safety and yield of ultrasound-assisted transthoracic biopsy performed by pulmonologists. *Respiration* 2004; 71: 519–522.
4 Gong Y, Sneige N, Guo M, Hicks ME, Moran CA. Transthoracic fine-needle aspiration *vs.* concurrent core needle biopsy in diagnosis of intrathoracic lesions: a retrospective comparison of diagnostic accuracy. *Am J Clin Pathol* 2006; 125: 438–444.

5 Greif J, Marmur S, Schwarz Y, Man A, Staroselsky AN. Percutaneous core cutting needle biopsy compared with fine-needle aspiration in the diagnosis of peripheral lung malignant lesions: results in 156 patients. *Cancer* 1998; 84: 144–147.

6 Keebler KM. Cytopathology techniques. *In*: Bibbo M, ed. Comprehensive Cytopathology. 2nd Edn. Philadelphia, WB Saunders, 1997; pp. 881–906.

7 Davenport RD. Rapid on-site evaluation of transbronchial aspirates. *Chest* 1990; 98: 59–61.

8 Diacon AH, Schuurmans MM, Theron J, *et al.* Utility of rapid on-site evaluation of transbronchial needle aspirates. *Respiration* 2005; 72: 182–188.

9 Conces DJ Jr, Schwenk GR Jr, Doering PR, Glant MD. Thoracic needle biopsy. Improved results utilizing a team approach. *Chest* 1987; 91: 813–816.

10 Austin JH, Cohen MB. Value of having a cytopathologist present during percutaneous fine-needle aspiration biopsy of lung: report of 55 cancer patients and metaanalysis of the literature. *AJR Am J Roentgenol* 1993; 160: 175–177.

Appendix H

Randomised comparison of two staining strategies for rapid on-site analysis of transbronchial needle aspirates.

Oral presentation at European Respiratory Society Meeting 2006, Munich. Europ Respir J 2006;28:535s

Citation: Europ Respir J 2006;28:535s

Randomised comparison of two staining strategies for rapid on-site analysis of transbronchial needle aspirates

Andreas H. Diacon, Michel M. Van den Heuvel, Coenraad F.N. Koegelenberg, Karen Brundyn, Mercia Louw, Colleen A. Wright, Chris T. Bolliger.

Introduction

Rapid on-site analysis (ROSE) improves yield and practical use of transbronchial needle aspiration (TBNA). This study compared two on-site staining strategies: 1) “Budget”, a simple, 30-second, cytoplasmatic stain (Diff-Quik) carried out and read by one person, and 2) “Luxury”, a sophisticated, 3-minute stain with better resolution of nuclear features (Rapid Papanicoleau), operated and read by two persons.

Method

We randomised 126 patients (55±15 years; 60% male) to TBNA with budget ROSE (B-ROSE, n=63) or luxury ROSE (L-ROSE, n=63). Sampling at a target site was terminated after 5 passes or upon two positive needle passes. ROSE results were compared to the final TBNA laboratory report.

Results

TBNA was positive in 99 (79%) of all patients. With 827 needle passes at 217 target sites we diagnosed lung carcinoma (81%), granulomatous disease (17%) and lymphoma (2%). False negative and false positive needle passes were more frequent with B-ROSE than with L-ROSE (27.2% vs 15.1%, p=0.01; 9.6% vs 5%, p=0.18), as well as false negative target sites (B-ROSE: 16.9%, L-ROSE 4.5%, p=0.02). No false positive sites occurred with either method. B-ROSE resulted in fewer bronchoscopies with positive ROSE (93% vs 100%, p=0.20).

Conclusion

Rapid Papanicoleau is superior to Diff-Quik for yield and accuracy of ROSE-TBNA.

Appendix I

List of other publications generated from this project

Other presentations and publications derived from this project

Articles

- Schubert P, Wright CA, Louw M, Brundyn K, Theron J, Bolliger CT, Diacon AH. Ultrasound Assisted Transthoracic Biopsy: Cells or Sections? Diagn Cytopathol 2005; 33:233-7.
- Diacon AH, Theron J, Schuurmans MM. Transthoracic growth of pleural malignant mesothelioma. Minerva. BMJ 2004; 328: 116.
  German translation of this article: Diacon AH. Steinharte Beule nach Thoraxdrainage. Medical Tribune 2004; 7:11.
- Diacon AH, Theron J, Bolliger CT. Transthoracic ultrasound for the pulmonologist. Curr Opin Pulm Med. 2005;11:307-12.

Invitations

- Speaker: Mediastinal staging of Lung Cancer. Oral presentation. South African Thoracic Society Meeting 2005, Sun City.
- Chair: Poster discussion session: Interventional pulmonology in clinical practice. European Respiratory Society congress, Munich, 2006.

Congress abstracts (oral presentations and posters)

- Diacon AH, Van den Heuvel MM, Koegelenberg CFN, Brundyn K, Louw M, Wright CA, Bolliger CT. Randomised comparison of two staining strategies for rapid on-site analysis of transbronchial needle aspirates. Oral presentation at European Respiratory Society Meeting 2006, Munich. Europ Respir J 2006;28:535s
- Diacon AH, Theron J, Brundyn K, Schubert P, Bolliger CT. Ultrasound assisted transthoracic biopsy: direct comparison of fine needle aspiration and cuttting needle biopsy. Oral presentation at European Respiratory Society Meeting 2005, Kopenhagen.

Europ Respir J 2005;26:318s

- Diacon AH, Theron J, Brundyn K, Louw M, Wright CA, Bolliger CT. Ultrasound assisted transthoracic biopsy: direct comparison of fine needle aspiration and cuttting needle biopsy. Oral presentation at South African Thoracic Society Meeting 2005, Sun City. SA Respiratory Journal 2005;11:60
- Diacon AH, Theron J, Brundyn K, Louw M, Wright CA, Bolliger CT. Ultrasound assisted transthoracic biopsy: direct comparison of fine needle aspiration and cuttting needle biopsy. Oral presentation at South African Thoracic Society Meeting 2005, Sun City. SA Respiratory Journal 2005;11:60
- Diacon AH, Theron J, Brundyn K, Louw M, Wright CA, Bolliger CT. Transbronchial needle aspirates: direct comparison of two handling methods. Poster presentation at the Asian Pacific Society of Respirology meeting 2005, Perth, Australia. Respirology 2005;10(S):A71
- Diacon AH, Schuurmans MM, Louw M, Wright CA, Bolliger CT. Practical value of rapid on-site evaluation of transbronchial needle aspirates. Poster presentation at the Asian Pacific Society of Respirology meeting 2005, Perth, Australia. Respirology 2005;10(S):A71
- Diacon AH, J Theron, M Louw, K Brundyn, CA Wright, CT Bolliger. Transbronchial needle aspirates: now many per target site? Poster presentation at the Asian Pacific Society of Respirology meeting 2005, Perth, Australia. Respirology 2005;10(S):A71
- Diacon AH, MM Schuurmans, J Theron, M Louw, K Brundyn, CA Wright, CT Bolliger. Transbronchial needle aspirates: now many per site? Poster presentation at the European Respiratory Congress, Glasgow, 2004. European Resp J. 2004;24:490S

- Diacon AH, Theron J, Brundyn K, Louw M, Wright CA, Bolliger CT. Transbronchial needle aspirates: direct comparison of two handling methods. Oral presentation at the South African Thoracic Society Congress, Durban, 2004. SA Respiratory Journal 2004;10:65
- Diacon AH, Schuurmans MM, Louw M, Wright CA, Bolliger CT. Practical value of rapid on-site evaluation of transbronchial needle aspirates. Poster presentation at the European Respiratory Congress, Vienna, 2003. European Respiratory Journal 2003; 22:58S
- Diacon AH, Schuurmans MM, Louw M, Wright CA, Bolliger CT. Practical value of rapid on-site evaluation of transbronchial needle aspirates. Poster presentation at the American Thoracic Society Meeting, Seattle, 2003. American Journal of Respiratory and Critical Care Medicine 2003; 167:A535
- Diacon AH, Schuurmans MM, Schubert P, Wright CA, Bolliger CT. Ultrasound assisted Tru-Cut biopsy of pleural tumors: A prospecitve analysis of 62 consecutive cases in a high incidence area of malignant mesothelioma. Poster presentation at the American Thoracic Society Meeting, Seattle, 2003. American Journal of Respiratory and Critical Care Medicine 2003; 167:A902
- Diacon AH, Schuurmans MM, Louw M, Wright CA, Bolliger CT. Practical value of rapid on-site evaluation of transbronchial needle aspirates. Oral presentation at the South African Thoracic Society Meeting, Cape Town, 2003. SA Respiratory Journal 2003; 9:125
- Diacon AH, Schuurmans MM, Theron J, Louw M, Wright CA, Bolliger CT. The right number of transbronchial needle aspirates. Oral presentation at the South African Thoracic Society Meeting, Cape Town, 2003. S A Respiratory Journal 2003; 9:126

- Diacon AH, Schuurmans MM, Schubert P, Wright CA, Bolliger CT. Ultrasound assisted cutting needle biopsy of pleural tumors. Oral presentation at the European Respiratory Society congress, Stockholm, 2002. European Respiratory Journal 2002; 21:594S
- Diacon AH, Schuurmans MM, Schubert P, Wright CA, Bolliger CT. Ultrasound assisted cutting needle biopsy of pleural tumors. Oral presentation at the South African Thoracic Society congress, Sun City, 2002. SA Respiratory Journal 2002; 8:67