Transbronchial Needle Aspirates*

Comparison of Two Preparation Methods

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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.

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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.