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Feasibility of Endobronchial Ultrasound Transbronchial Needle Aspiration for Massively Parallel Next-Generation Sequencing in Thoracic Cancer Patients

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Abstract

Introduction—Next-generation sequencing (NGS) allows for the identification of a growing number of therapeutic and prognostic molecular targets. However, NGS typically requires greater quantities of DNA than traditional molecular testing does. Endobronchial ultrasound transbronchial needle aspiration (EBUS-TBNA) is a minimally invasive procedure used to sample central thoracic lesions, but it is not well established whether this technique provides sufficient material for NGS.

Methods—We performed a retrospective review of EBUS-TBNA at our institution (3/1/14-9/28/16). NGS was performed using a comprehensive hybrid-capture based assay (MSK-IMPACT) that detects >340 gene mutations. Samples found to be diagnostic for malignancy and for which MSK-IMPACT had been attempted were identified. Pathologic and clinical data were obtained from the medical record, and the results of MSK-IMPACT were examined.

Results—In total, 784 EBUS-TBNA procedures were performed during the study period. MSK-IMPACT was requested for 115 malignant samples and was successful for 99 (86.1%), identifying an average of 12.7 mutations at a mean coverage depth of 806X. NGS was performed on paraffin-embedded cell blocks in 93 cases (93.9%) and on cell-free DNA in needle rinse fluid in 6 cases. The success rate of the assay improved significantly from the first third of cases (76.3%), to 92.3% for the final one-third of cases ($p < 0.05$).

Conclusions—EBUS-TBNA reliably provided adequate tissue for hybrid capture NGS, and is a suitable option for comprehensive NGS testing in patients with thoracic malignancies.

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Keywords

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1. Introduction

Next-generation sequencing (NGS) is an important tool in precision oncology, allowing improved prognostication and the identification of a growing number of clinically validated and investigational therapeutic molecular targets.[1] In non-small cell lung cancer (NSCLC)and , more specifically, lung adenocarcinoma, targeted therapies for *EGFR* mutations and *ALK-EML4* or *ROS1* rearrangements now compose part of the standard of care. [2–5] In addition, several alterations found in a subset of patients such as *BRAF* V600E, *RET* fusions, *MET* exon 14 splice site mutations , and *ERBB2* amplification or activating mutations may predict response to targeted therapies, such as those already approved for other tumor types or those approved on the basis of preclinical studies. [6–13]Several studies have identified *KRAS* mutations as an important negative prognostic marker, [14,15]and , more recently, targeted approaches have been described for *KRAS*-mutant NSCLC.[16,17] In this setting, comprehensive genomic profiling using NGS allows for the identification of a wide spectrum of genomic alterations, resulting in personalized cancer treatment of an increasing number of patients, as well as the inclusion of patients in clinical trials, including so-called basket trials, organized by oncogenic driver alterations instead of by tissue of origin. The results of the recently reported ProfILER trial demonstrate that large -scale NGS screening of cancer patients is both feasible and associated with a survival benefit. [18]

Memorial Sloan Kettering Cancer Center (MSK) has developed a next-generation massively parallel molecular sequencing assay known as Memorial Sloan Kettering–Integrated Mutation Profiling of Actionable Cancer Targets (MSK-IMPACT). This hybrid capture-based NGS assay detects point mutations, small insertions/deletions, copy number alterations, and selected fusions in >340 cancer-related genes.[19,20] Of these, 19 are clinically validated, while the rest are considered investigational. A complete description of the panel has been published.[19] Such a comprehensive assay is well-suited to the context of NSCLC, which includes a wide range of genomic alterations. In a recent study, 37% of lung cancer patients received matched treatment after molecular profiling using the MSK-IMPACT assay.[21] Compared with amplicon-based assays, however, hybrid capture-based assays, such asMSK -IMPACT, generally require a higher input of nucleic acids. The preferred source of material for molecular testing of NSCLC, in both presurgical and advanced-stage settings, is small specimens acquired with minimal invasiveness, including cytologic samples. In this setting, a potential limitation of NGS-based assays for comprehensive genomic profiling is the need for more DNA than would be required for traditional molecular assays, which perform well using cytologic samples without the requirement for major technical adjustments.[22,23]As such, there is a debate regarding whether minimally invasive, fine -needle aspiration biopsy techniques are appropriate when comprehensive NGS assays are planned.

Endobronchial ultrasound transbronchial needle aspiration (EBUS-TBNA) is a minimally invasive procedure for sampling mediastinal and pulmonary lesions. It is used to diagnose mediastinal lymphadenopathy and central lung masses, to stage mediastinal lymph nodes in patients with NSCLC and other malignancies, and to acquire tissue in the setting of malignant disease progression despite medical therapy. EBUS-TBNA is generally performed as an outpatient procedure and may be done with or without the use of general anesthetic. Morbidity is minimal and compares favorably with that of more-invasive techniques, such as mediastinoscopy or thoracoscopy.[24] The reported sensitivity, specificity, and diagnostic accuracy of nodal staging for NSCLC are all >90% [25,26] and have been shown to be comparable or even superior to those of surgical staging.[27] The utility of EBUS-TBNA for molecular testing can be further improved with the use of rapid on-site cytologic evaluation (ROSE), which allows a cytopathologist to provide real-time feedback regarding specimen adequacy during the EBUS procedure.[28] Several studies have confirmed the feasibility of using EBUS-TBNA samples for traditional molecular assays such as EGFR, ALK and KRAS testing,[22,29,30] and even small multi-gene panels,[31] but the suitability of the technique for NGS assays, that can test for hundreds of genes, has only recently been established.[32] The Papanicolaou Society of Cytopathology guidelines discuss the use of EBUS-TBNA for NGS, but the evidence supporting this guideline consists of a single, five-patient series including both EBUS and CT guided fine-needle samples and a 27-gene NGS panel.[33,34] Concerns remain regarding the ability of EBUS to reliably obtain sufficient material for NGS, leading some oncologists to still prefer surgical specimens or core biopsies. Since only limited evidence exists to support the performance of comprehensive NGS panels on EBUS-TBNA specimens [32], we sought to address the question of whether EBUS-TBNA can reliably provide sufficient material for large hybrid capture NGS.

2. Materials and Methods

We performed a retrospective review of a prospectively maintained thoracic surgery database, including all patients who underwent EBUS-TBNA by the Thoracic Surgery Service at MSK between March 1, 2014, when MSK-IMPACT was introduced for EBUS samples, and September 28, 2016. A waiver for the use of patient medical records was obtained from the MSK Institutional Review Board. Samples found to be positive for malignancy that had undergone MSK-IMPACT testing were identified. The results of the MSK-IMPACT assay were examined. Samples that either contained borderline tumor content or for which the assay was not successful (see below) were re-reviewed pathologically. Student's *t* test, Fisher's exact test, and the χ^2 test were used to identify statistical differences between groups. *P* values refer to Student's *t* test, except where noted.

2.1 EBUS-TBNA Technique

In our group, all EBUS-TBNA procedures are performed under general anesthesia with either laryngeal mask airway or single lumen endotracheal intubation. An Olympus BF-UC180F EBUS (Olympus, Tokyo, Japan) scope is used, with a 21- or (infrequently) 22-gauge EBUS needle. ROSE is used to assess specimen adequacy in all cases. Based on a recent quality assurance survey of our surgeons, typically, 3 to 5 passes are made in each

node, guided by ROSE assessment of adequacy, with approximately 10 to 15 strokes of the needle within the node per pass.

2.2 Genomic and Pathologic Sample Analysis

Cell block material was obtained from CytoLyt-fixed, paraffin-embedded 5-micron-thick cell block sections on glass slides (range 5 to 21 slides/case). For all cases, 20 recuts were prepared for DNA extraction and at least 1 hematoxylin and eosin-stained (H&E) section was reviewed for assessment of specimen adequacy and estimation of tumor fraction. Macrodissection, by circling the area of enriched tumor on the slide, was performed when needed. Cases were rejected if <10% of tumor content was present (i.e., if tumor cells represented <10% of total nucleated cell cellularity, which included lymphocytes, bronchial cells, and stromal cells based on the H&E review). For positive samples in which cell blocks failed to capture a sufficient number of tumor cells, molecular testing was performed on cell-free DNA (cfDNA) from the needle rinse fluid in CytoLyt. Retrieving the cfDNA from the CytoLyt fluid was achieved by collecting the supernatant fluid present after cells were centrifuged from needle rinse fluid to form a cell block. The details of this procedure were described recently.[35,36]

Sequencing of tumor and blood DNA was performed using the clinically validated MSK-IMPACT assay.[20] In brief, DNA was extracted using the DNeasy Tissue KIT (Qiagen, Valencia, CA), with either Citrasolv or (starting in March 2016) mineral oil for deparaffinization.[34] After elution, quantification was performed using a Qubit DNA high-sensitivity assay kit (Life Technologies, Carlsbad, CA). Samples for which 50 ng of DNA input was reached were processed; those below this cutoff were tested on alternative platforms (mass spectrometry genotyping [Sequenom, San Diego, CA]) downstream. Fragment analysis of exon 19 and 20 of *EGFR* and exon 20 of *ERBB2* was performed before MSK-IMPACT testing on all lung adenocarcinoma samples. DNA was submitted to shearing and processed to generate bar-coded libraries, pooled, and subjected to exon capture using custom-designed probes. Captured DNA fragments were sequenced on an Illumina HiSeq2500 system before being subjected to the bioinformatics analysis pipeline. In this assay, matched normal DNA from blood is simultaneously processed with tumor DNA for all samples, allowing identification and filtering out of single-nucleotide polymorphisms.

Over the course of the study, the number of genes included in the assay was increased from 341 to 469. Most samples (88%) were processed with the intermediate version (including 410 genes); only 3% were processed with the initial version (including 341 genes). All versions included a total of 19 genes with full clinical validation for which the cutoff for variant calling was set at 2%; it was set at 5% for all other genes in the panel (called “investigational”) meaning that mutations are reported only if they reached this frequency. Tumor samples were required to be sequenced to 200X coverage.

At the time of reporting, samples meeting the assay performance criteria (i.e., >10% proportion of tumor cells by visual estimate and >50 ng of extracted DNA) but in which tumor cells represented <20% of the total nucleated cells within the sample were flagged as having borderline tumor content, to highlight the risk of false-negative results in mutations,

copy number alterations, and structural variants called. Samples submitted for the MSK-IMPACT assay were thus classified into 3 categories for the purposes of this study: (1) fully adequate, (2) borderline tumor content, and (3) unsuccessful assay (inadequate sample or failure of the MSK-IMPACT assay).

2.3 Molecular Diagnostics Review

Parameters of tumor and DNA quantification and assay performance were reviewed for samples from each category (fully adequate, borderline tumor content, and unsuccessful assay). Preanalytical parameters reviewed included estimation of tumor cell fraction (tumor content), number of slides available for extraction, and DNA extraction yield (concentration and volume). In addition, NGS quality-control metrics were collected and used as a proxy for DNA quality, including median coverage, peak insert size, duplication rate, and percentage of read trimming. Genomic data collected for each sample included the number of variants, copy number alterations, and structural variants called, as well as the highest mutation frequency found in the tumor. In addition, the rate of mitogenic driver detection was determined for lung adenocarcinoma, since this was by far the most commonly sampled tumor type in the cohort. For this purpose, known recurrent hot-spot activating mutations in *EGFR*, *ERBB2* (exon 20 insertion and activating mutations), *KRAS*, *NRAS*, *MAP2K1/* *MAP2K4*, and *PIK3CA*, as well as *MET* exon 14 splicing mutations and *ALK-*EML4**, *ROS1*, or *RET* fusions, were considered.

3. Results

In total, 784 EBUS-TBNA procedures were performed by the Thoracic Surgery Service at MSK during the study period. Malignancy was identified in 315 patient samples, and MSK-IMPACT was ordered for 115 of those samples (Fig. 1). (MSK-IMPACT is not ordered on biopsy specimens when a complete resection specimen is ultimately obtained later). Each sample represents a unique patient, as no patient was tested more than once. Although many patients had >1 site sampled by EBUS-TBNA during a given procedure (e.g., multiple separate mediastinal lymph nodes), the material from only 1 site was ultimately used for MSK-IMPACT testing. Among tested tumors, the most common histologic subtype was lung adenocarcinoma (79 [68.7%]), followed by poorly differentiated carcinoma (including NSCLC not otherwise specified [16 (13.9%)]), lung squamous cell carcinoma (8 [7.0%]), small cell lung cancer (5 [4.3%]), neuroendocrine tumor (2 [1.7%]), and mesothelioma, melanoma, prostate carcinoma, sarcoma, and breast carcinoma (1 of each [0.9%]). The source of DNA extraction for NGS was paraffin-embedded cell blocks in 93 cases (93.9%) and cfDNA in needle rinse fluid in 6 cases where the cell block was insufficient. All 6 cases performed from this material were among the last half of the cases in the series.

The assay was completed successfully on 99 of the 115 samples (86.1%), identifying an average of 12.7 mutations at a mean coverage depth of 806X. A mean of 1.3 mutations (range 0–5) in the validated panel and 11.4 mutations (range 0–57) in the investigational panel were identified. Most of these samples (87 [87.9%]) had at least 1 mutation detected in the clinically validated panel. *EGFR* mutations were detected in 19 samples (19.2%) overall

and 12/75 lung adenocarcinomas (16%), and *ALK* rearrangements were detected in 4/75 of lung adenocarcinomas (5%).

Specimen quantity and quality were judged to be fully adequate for 82 of the 99 successful samples (82.8%). On average, 13.9 genomic alterations were detected, including 1.3 in the clinically validated group and 12.6 in the investigational group, at an average mean coverage depth of 837X. In 17 samples (17.2 %), the MSK-IMPACT assay was completed successfully but the samples were found to have border line tumor content, although each of these had at least 1 genomic alteration detected.

Compared with fully adequate samples, borderline tumor content samples had fewer average genomic alterations detected (6.8; $p=0.01$), including, on average, 1.2 clinically validated mutations ($p=0.44$) and 5.6 investigational mutations ($p=0.02$) in each sample and an average mean coverage depth of 657X ($p=0.05$). However, the proportion of candidate oncogenic driver alterations detected in lung adenocarcinoma samples in this subset (7/12 [58.3%]) was not significantly different from that in the fully adequate group (47/63 [74.6%]; Fisher's exact test, $p=0.30$). There was no significant difference in preanalytical factors, such as the number of slides extracted or DNA yield/input, between fully adequate and borderline tumor content samples (table available as supplement). In addition, the estimated tumor content (by review of the hematoxylin and eosin-stained slide before testing) was, as expected, lower in borderline tumor samples than in fully adequate samples (15% vs 40%; $p<0.0001$). This difference by tumor content was also reflected in a lower mutation frequency (proportion of mutant allele detected in the tumor) in the borderline tumor group (19% vs 47%; $p<0.0001$). Cytologic review of the cell block sections was performed for all samples with borderline tumor content, and in all 17 cases the cell block sections contained adequate tumor cellularity but also contained abundant benign elements, resulting in a relative borderline tumor percentage. Finally, sequencing coverage (table available as supplement) and other metrics used as an indirect way to assess DNA quality (peak insert size, duplication rate, and percentage of read trimming) were not different between categories (data not shown). Overall, these parameters confirm the good performance of the assay to detect clinically relevant genomic alterations in EBUS-FNA samples, including in borderline samples with low tumor content.

The overall success rate of the assay improved over time (Fig. 2). In the earliest one-third of cases, 29 of 38 (76.3%) were successful (25 fully adequate, 4 borderline tumor content), whereas in the most recent third of cases, 36 of 39 (92.3%) were successful (29 fully adequate, 7 borderline tumor content) (χ^2 , $p=0.02$).

In total, 16 cases were categorized as unsuccessful (13.9%) (Fig. 3). Cytologic review of the cell block sections for these samples revealed two main issues: (1) the sections were cellular but contained abundant benign elements (lymphocytes, bronchial epithelium, etc.), resulting in a low tumor percentage (12 cases), or (2) the cell block sections were paucicellular, containing only a scant amount of tumor cells (3 cases). In 13 of the 16 unsuccessful cases (all lung cancer cases), MSK-IMPACT was not attempted. Eight of these cases were rejected out of hand before extraction (<10% tumor cells) for 3 of these, fragment analysis for exon 19 and 20 of *EGFR* and exon 20 of *ERBB2* was eventually performed. In the other 5 cases,

DNA was extracted but MSK-IMPACT was not attempted owing to insufficient DNA yield (<50 ng). Sequenom was successfully performed as an alternative in 4 of these cases; *EGFR*/*ERBB2* fragment analysis was performed in the other. Two of the remaining unsuccessful cases had technical failures: low coverage (<100x) and nonhost contamination. Alternative multiplex testing by Sequenom was performed in both cases, and *KRAS* mutation was detected in 1 case. The final case in this category had no genomic alterations detected among the genes covered by the assay, although it was deemed to have performed well on the basis of assessment of tumor content and quality-control metrics. Even when a sample is judged adequate by ROSE, this does not guarantee an adequate cell block will be obtained. Cytologic review of the cell block sections in this case found adequate tumor content. Overall, of the 16 samples that were unsuccessful for MSK-IMPACT testing, 10 underwent molecular testing by an alternative method (Fig. 3). In total, 109 of the 115 included samples (94.8%) were adequate for some form of molecular testing.

4. Discussion

NGS assays such as the MSK-IMPACT are important tools to identify patients who may benefit from approved targeted therapies for lung cancer (such as *EGFR* and *ALK* tyrosine kinase inhibitors), to improve prognostication, and to select patients for an ever-growing number of clinical trials in molecular oncology. To our knowledge, this study is the largest series, and one of only two yet published, to demonstrate the feasibility of using EBUS-TBNA to obtain adequate tissue for NGS in a panel as comprehensive as MSK-IMPACT. Existing guidelines that support the use of EBUS-TBNA samples for NGS are based almost exclusively on data from studies on CT-guided fine-needle aspirates.[33,34,37–40] EBUS-TBNA has several advantages over CT-guided needle biopsy. EBUS allows taking multiple passes from multiple sites without increasing the morbidity of the procedure and can therefore be used to provide complete staging of the mediastinum concurrently at the same time as making a diagnosis, which cannot be done with CT-guided biopsy. CT-guided needle biopsy is limited in the number of passes that can be made, and the number of sites that can be biopsied at one time. Unlike CT-guided biopsy, EBUS carries little or no risk of pneumothorax.

This study demonstrates that MSK-IMPACT, a targeted but comprehensive hybrid capture-based NGS assay, can be performed with a high rate of success using EBUS-TBNA samples that are diagnostic for malignancy. More than 85% of samples were successfully analyzed by the MSK-IMPACT, and it was possible to identify candidate driver alterations in most of the samples with borderline tumor content. Borderline samples had lower tumor content than fully adequate samples, likely explaining the lower number of mutations found in the investigational panel. Indeed, the threshold for mutation calling was higher for genes in the investigational panel than for those in the clinically validated panel (5% vs 2%). As we found that both the estimated tumor fraction and the highest mutation frequency detected in tumor were lower in borderline samples, the likelihood of detecting subclonal mutations in these samples was therefore decreased. Clinically validated genes in the panel included common driver mutations (per example *EGFR* L858R), which are more likely to be clonal and can be detected even at low levels of tumor content, owing to their expected higher frequency in tumors. In our cohort, we observed a similar proportion of clinically validated genes

between the fully adequate and borderline groups, as well as a high rate of driver mutations in lung adenocarcinoma samples in both groups. No other parameters were analyzed, such as sequencing coverage, that could explain the lower number of investigational mutations found in the borderline samples. In addition, this review of metrics showed that the assay performed well with cytologic specimens from EBUS, even in the context of borderline tumor content.

In an attempt to identify any technical or anatomic issues that may have contributed to poor sample quality, the clinical records of the 33 patients with less than fully adequate samples (17 borderline and 16 unsuccessful) were also examined. No issues could be consistently identified. Contemporaneous computed tomography scans of the chest were available for 32 of these patients 31 had enlarged target nodes by computed tomography criteria. Contemporaneous positron emission tomography scans were available for 26 patients, all of which showed positron emission tomography avid target nodes. The operative report contained a description of the ultrasonic findings in 24 cases 23 of these had large nodes on ultrasound.

Whereas the success rate seen across the entire study was quite good, it actually improved over time, to 92.3% for the last third of samples tested. We speculate that this may be related partly to possible refinements in EBUS-TBNA technique and increased experience with ROSE in the operating room. In addition, DNA extraction techniques at our institution evolved during the study period, including the introduction of mineral oil as a solvent, which, in our experience, has been associated with improved extraction yield.[35] Last, our cytology division recently modified its cell block preparation technique, and this change has substantially improved the sufficiency of cytologic samples for molecular studies.[36] These improvements in processing technique may have contributed to the improved success rate seen over time. Improvements in the technical proficiency of performing EBUS-TBNA by our surgeons may also have occurred during the study period, resulting in better tissue acquisition and more successful MSK-IMPACT assays.

An important finding in this study was that, in the vast majority of cases, failure to perform the assay resulted from aspirates containing an admixture of lymphocytes and tumor cells. This “contamination” is related to the nature of the aspiration from a lymph node that is, it is typical for a needle to traverse both the tumor and benign lymphoid areas. Conversely, we found that only in rare instances was the inability to perform molecular studies related to the overall paucicellularity of a sample.

Several factors may have contributed to the high success rate of molecular studies using EBUS samples in this study. First, every EBUS procedure at our institution is accompanied by live on-site assessment by a cytopathologist, which allows the surgeons to perform multiple additional passes of lymph nodes that are assessed as positive. Second, the ability to use cfDNA from needle rinse fluid for molecular studies a new process in our cytopathology laboratory has increased the success rate of molecular testing in samples with scant tumor content, though this technique was only used in a small number of samples in this series. Further studies may compare the use of cell block tissue and cfDNA from the same specimen to confirm the efficacy of this technique. Reassuringly, even among the few

samples deemed unsuccessful for the assay, most were adequate for conventional molecular testing with PCR and/or Sequenom, allowing for 95% of all included samples to be tested for candidate drivers by molecular methods, either NGS based or traditional.

The study was restricted to EBUS-TBNA procedures performed by the Thoracic Surgery Service at MSK. This allowed for the inclusion of a relatively homogenous set of EBUS techniques and technical factors, such as the use of general anesthesia, ROSE, and consistent equipment, which may vary among other practitioners performing the procedure. The high rate of success seen in our series likely reflects the optimized conditions under which the procedures were performed. All of the surgeons included in this study learned EBUS technique while in thoracic surgery practice, and typically perform 5–10 procedures per month.

This study may be limited by the method used to identify cases for inclusion in the retrospective review. Cases were identified by the presence of a diagnostic molecular pathology report specifically denoting 1 of 3 possible outcomes from the MSK-IMPACT assay: positive for any genomic alteration, negative for all genomic alterations, or insufficient sample. This identifies only patients who had a sample positive for malignancy for which the MSK-IMPACT assay was attempted. Patients with nondiagnostic samples (i.e., those insufficient to rule malignancy in or out) would not be captured, as these samples would not be sent on for MSK-IMPACT testing. Nondiagnostic samples reflect a technical failure of the EBUS-TBNA procedure in general that is unrelated to the MSK-IMPACT assay. The sensitivity and specificity of EBUS-TBNA, both >90%, have been established elsewhere, and we did not attempt to replicate this work. The high success rate seen in this study should be interpreted to reflect the success of the MSK-IMPACT assay using EBUS-TBNA samples that were diagnostic for malignancy.

Future studies will determine the effect that MSK-IMPACT testing of EBUS-TBNA samples had on patient care in this cohort. However, the demonstration of a survival benefit in patients undergoing large-scale NGS screening in the recent ProfILER study[18] underscores the importance of molecular characterization and NGS for patients with cancer. The ability to obtain a molecular profile of disease using an endoscopic approach (EBUS-TBNA) provides a valuable tool in the care of patients with thoracic malignancies. This is possible, in part, because of the ability of the MSK-IMPACT assay to perform NGS with high sensitivity in samples with small quantities of DNA, even those that may be considered to have borderline tumor content.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations

cfDNA	cell-free DNA
EBUS-TBNA	endobronchial ultrasound transbronchial needle aspiration
MSK	Memorial Sloan Kettering Cancer Center
NGS	next-generation sequencing
NSCLC	non-small cell lung cancer
ROSE	rapid on-site cytology evaluation

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Highlights

- Next-generation sequencing is gaining importance in treating thoracic malignancies.
- However, NGS requires high DNA input for hybrid capture assays.
- EBUS-TBNA reliably provided adequate specimens for NGS.
- Success improved over time, possibly due to surgical technique or tissue processing.

Figure 1. CONSORT diagram of endobronchial ultrasound transbronchial needle aspiration (EBUS-TBNA) procedures performed during the study period. Numbers refer to individual EBUS procedures

Figure 2.
Success rate of the MSK-IMPACT assay over time, by third of cases included in the study.

Figure 3.
Reasons for unsuccessful MSK-IMPACT assay and alternative molecular assays performed.
cfDNA, cell-free DNA.