Adequacy of Core Needle Biopsy Specimens and Fine-Needle Aspirates for Molecular Testing of Lung Adenocarcinomas

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ABSTRACT

Objectives: Molecular testing of lung adenocarcinomas for epidermal growth factor (EGFR) mutations and an anaplastic lymphoma kinase (ALK) translocation is important to guide directed therapy with tyrosine kinase inhibitors. The goal of this study was to determine whether transthoracic computed tomography–guided core needle biopsy (CNB) and fine-needle aspiration (FNA) biopsy specimens were equally suitable for molecular testing.

Methods: We determined the percentage of 52 CNB and 120 FNA specimens that contained sufficient paraffin-embedded tumor tissue for EGFR, KRAS, and ALK testing over a period of 2 years. We correlated sample sufficiency with the sampling method, tumor size, biopsy operator, pathologist assessing the adequacy of the sample, and the number of FNA passes performed.

Results: Univariate analysis showed that CNB specimens provided a significantly higher number of samples sufficient for molecular testing than did FNA specimens (67% vs 46%; \( P = .007 \)) and that one operator achieved a significantly higher percentage of sufficient FNA specimens. Binomial logistic regression found sufficiency of FNA samples to correlate with tumor size (\( P = .015 \)) but not operator.

Conclusions: When paraffin-embedded tissue is used for molecular testing of lung cancer, CNB specimens are more likely than FNA specimens to provide adequate tissue for molecular testing. Obtaining a sufficient FNA specimen depends on the tumor size and the individual performing the biopsy.

ANCILLARY

Upon completion of this activity you will be able to:
• describe recommended molecular testing to select lung cancer patients for targeted therapies.
• discuss advantages and disadvantages of computed tomography–guided core needle biopsy and fine-needle aspiration for sampling of lung cancer for molecular testing.
• recommend sample type and cellularity optimal for molecular testing for targeted therapy in lung cancer.

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abnormalities in cancer will likely lead to the identification of additional therapeutic targets in the near future.

Molecular testing of lung adenocarcinomas requires procurement of adequate involved tissue. Specimens suitable for testing include formalin-fixed, paraffin-embedded (FFPE) tissue and fresh (unfixed), frozen, and alcohol-fixed tissue. When using cytology samples, cell block preparations are preferred over direct smears. Some have successfully employed for testing needle rinses directly or tumor cells taken off direct smears or liquid-based preparations. Recommended testing methods include polymerase chain reaction (PCR)-based assays for EGFR mutations and fluorescence in situ hybridization (FISH) assays for ALK testing.

Surgical lung cancer resections usually offer abundant tumor tissue for testing. However, advanced lung cancers often do not undergo surgical resection. Consequently, pathologists routinely find themselves confronted with requests to perform molecular testing on small biopsy specimens obtained by computed tomography (CT)-guided core needle biopsy (CNB) or fine-needle aspiration (FNA) biopsy specimens.

Although cytology samples are considered acceptable specimens for molecular testing, we found in our practice an undesirably high rate of FNA specimens that were suitable for rendering a diagnosis but insufficient for molecular testing. This prompted us to assess the adequacy for EGFR and ALK testing of CNB and FNA specimens over a period of 2 years. We report here the adequacy of each method to obtain material sufficient for molecular testing. We also discuss factors associated with successful and unsuccessful biopsies.

Materials and Methods

The anatomic pathology laboratory information system of the University of Pittsburgh Medical Center was queried for all CT-guided lung FNAs and CNBs between July 2011 and June 2013 for which a diagnosis of either lung adenocarcinoma or non–small cell carcinoma, favor adenocarcinoma, was rendered. Only CT-guided transthoracic biopsy specimens were included. Biopsy procedures were performed at different sites in our multihospital system and regional hospitals. Diagnoses were established using current guidelines. Rapid on-site assessments of diagnostic adequacy were performed for 118 (98%) of 120 of the FNA specimens. Molecular testing was ordered reflexively on cases with the above diagnoses. For a minority of procedures, testing had been performed previously or was not needed based on clinical circumstances. These cases were excluded from the study. Most biopsy specimens represented initial sampling of a newly found lung mass. A small minority may have represented intrapulmonary metastases or recurrences. Two patients underwent FNA twice on different dates with separate attempts at molecular testing. Operators chose the biopsy method based on their personal preference. Six patients had CNBs and FNAs performed during the same procedure because the FNA was used for targeting the tumor or the pathologist performing the on-site assessment requested a CNB and the operator felt it was safe to obtain it. In one case, both CNB and FNA were successfully tested; in one case, neither CNB nor FNA were sufficient; in one case, molecular testing was attempted on both samples, but only the core was sufficient; and in the remaining three cases, only the CNB specimens were tested successfully and the FNA specimens excluded from the study. This study was approved by the University of Pittsburgh Institutional Review Board.

CNBs were performed using 18- to 22-gauge spring-loaded core biopsy needles with or without a coaxial introducer needle. FNAs were performed using 22- to 25-gauge spinal or Chiba-type needles, usually without a coaxial introducer. Operators occasionally employed coaxial introducer needles for FNAs, usually in cases in which FNA was followed by CNB. Both CNB specimens and needle rinses from FNA specimens were fixed in neutral buffered 10% formalin. FNA material not used to prepare cytology smears on glass slides was spun down after fixation and the pellet placed in HistoGel (American MasterTech, Lodi, CA) before processing. After processing and paraffin-embedding using routine histology procedures, histotechnologists prepared 26 sections of 4 μm thickness. Levels 1 and 6 of FNA cell blocks and levels 1, 6, and 21 of CNB specimens were stained with H&E and sent to the attending pathologist for diagnosis. The remaining levels were used as outlined in Figure 1. Case pathologists were responsible for assessing whether the number of tumor cells in the paraffin-embedded tissue was sufficient based on the criteria below. If borderline cases were forwarded to the laboratory, a molecular pathologist reviewed the case and made a final decision.

For ALK FISH testing, a pathologist circled whole-slide recuts, and those circled areas were used for signal counting. Specimens with fewer than 100 tumor cells by manual counting were rejected. FISH was done on FFPE tumor tissues using a break-apart probe to the ALK gene (Vysis LSI ALK Dual Color, Break Apart Rearrangement Probe; Abbott Laboratories, Abbott Park, IL) per the manufacturer’s instructions. Sixty cells were scored. FISH-positive cases were defined as more than 15% split signals in tumor cells.

For PCR-based EGFR and KRAS testing, a minimum of 300 tumor cells on the H&E slide were accepted for analysis, a number that others have also found to represent a suitable sample. Tumor cells were manually microdissected by using a dissecting microscope. Care was taken that the
dissection target contained at least 50% tumor cells. DNA was isolated using standard laboratory procedure. For the detection of mutation, DNA was amplified with primers for exons 2 and 3 of the KRAS gene and exons 18 to 21 of the EGFR gene. Then, PCR products underwent fluorescence-based Sanger cycle sequencing in the sense and antisense directions using the BigDye Terminator version 3.1 cycle sequencing kit on the ABI 3130 (Applied Biosystems, Foster City, CA) according to the manufacturer’s instructions. The sequences were analyzed using Mutation Surveyor software (SoftGenetics, State College, PA). Each case was classified as positive or negative for the EGFR and KRAS mutations based on the sequencing results.

The outcome measures were (1) the percentage of FNA specimens on which molecular testing was successful and (2) the percentage of CNB specimens on which molecular testing was successful. Successful molecular testing was defined as the ability to report the presence or absence of (a) EGFR mutation, (b) KRAS mutation, and (c) ALK translocation. In response to the perceived high rate of unsatisfactory samples for molecular testing, a procedure change halfway through the study period encouraged pathologists present at an FNA procedure to request two additional needle passes solely for the cell block preparation once the biopsy specimen was felt to be adequate for a diagnosis of malignancy. We compared the primary outcomes before and after this change was implemented.

The following data were collected for each procedure: person performing the biopsy, tumor size, pathologist assessing adequacy of the sample at the time of biopsy, and number of FNA passes performed and number of additional FNA passes for the cell block preparation after the sample was deemed diagnostic during the on-site assessment. Means, medians, and standard deviations were calculated using Microsoft Excel (Microsoft, Redmond, WA). Statistical associations were tested with Pearson $\chi^2$ tests and binomial logistic regression using SPSS version 19 (SPSS, Chicago, IL). $P$ value of .05 or less was considered statistically significant.

**Results**

During the study period, 120 FNA and 52 CNB specimens were diagnosed as either lung adenocarcinoma or non–small cell carcinoma, favor adenocarcinoma, and eligible for molecular testing. On 62 FNA (52%) and two CNB (4%) specimens, testing was not ordered upfront by the case pathologist because the number of tumor cells was insufficient. We found that 35 (67%) of 52 CNB specimens and 55 (46%) of 120 FNA specimens contained sufficient tumor for molecular testing that allowed determination of EGFR and KRAS mutation status as well as ALK translocation status. Of the 65 inadequate FNA specimens, seven (11%) were sufficient for ALK FISH but not EGFR analysis, while another seven (11%) were sufficient for EGFR analysis but not ALK FISH. Image 1 illustrates two examples of such FNA specimens that were insufficient for one testing modality but not the other.

CNB specimens were significantly more likely to obtain sufficient material compared with FNA specimens ($P = .007$). Successful molecular testing of CNB specimens was not associated with tumor size or operator in a binomial logistic regression model.

The mean and median number of FNA needle passes performed for both adequate and inadequate biopsy specimens are shown in Table 1. After implementing a policy to collect two additional FNA passes for the cell block preparation, the success rate for molecular testing on FNA material increased from 45% to 48%. Although the mean number of FNA passes was higher for FNAs that allowed for successful molecular testing, we found no statistical correlation between adequacy of FNA samples for molecular testing and the number of FNA passes performed ($P > .5$).

Eleven different operators performed FNA biopsies during the study period. The two busiest individuals performed
Cell block preparations from two fine-needle aspirates that were insufficient either for EGFR/KRAS testing (A-C) or ALK testing (D-F). Low-power views highlight the paucity of tumor cell groups (arrows). A, D, Original sections evaluated by the pathologist for establishing a diagnosis. B, Recut section containing insufficient material for EGFR and KRAS analysis. C, Successful fluorescence in situ hybridization (FISH) testing for ALK. E, Recut section for successful EGFR/KRAS testing. F, Recut section showing insufficient material for ALK FISH. (A, B, H&E, ×40; C, in situ hybridization for ALK as described in the Materials and Methods, ×400; D, E, H&E, ×100; F, H&E, ×200).
Table 1

Comparison of the Success Rate and the Number of FNA Passes Performed Before and After Obtaining Two Additional Passes for Cell Block Preparation

<table>
<thead>
<tr>
<th>FNA Passes</th>
<th>Molecular Testing Successful, %</th>
<th>No. of FNA Passes When FNA Was Sufficient for Molecular Testing</th>
<th>No. of FNA Passes When FNA Was Insufficient for Molecular Testing</th>
</tr>
</thead>
<tbody>
<tr>
<td>Before obtaining two additional FNA passes</td>
<td>45</td>
<td>3.2 ± 1.1</td>
<td>3.7 ± 1.3</td>
</tr>
<tr>
<td>After obtaining two additional FNA passes</td>
<td>48</td>
<td>4.0 ± 1.3</td>
<td>3.7 ± 1.3</td>
</tr>
</tbody>
</table>

FNA, fine-needle aspiration.

47 and 42 FNAs, respectively; five others performed more than one. Performance of FNA operators with regard to obtaining aspirates sufficient for molecular testing is shown in Table 1. When comparing different operators performing FNAs, one operator was significantly associated with obtaining sufficient FNA samples in univariate analysis ($P = .017$). However, multivariate analysis of FNAs showed successful molecular testing to be associated with tumor size ($P = .015$) but not the operator ($P = .61$). The regression line of the multivariate model suggests that in our setting, FNA specimens of lung tumors larger than 3.5 cm had a greater than 50% chance of containing sufficient material for molecular testing Figure 2.

There was no association between successful molecular testing and individual pathologists performing adequacy assessments of the sample at the time of biopsy.

Discussion

Molecular testing of adenocarcinomas is important to identify driver mutations of lung cancer and enable oncologists to select proper treatment. Our experience suggests that CT-guided CNBs are superior to CT-guided FNAs when molecular testing of adenocarcinoma is desired.

Several studies have addressed the adequacy of small biopsy specimens for molecular testing, yet to our knowledge, none to date have compared the adequacy of CNBs and FNAs for EGFR and ALK testing in the same environment. Ferretti et al. report success rates for CNBs of 72% before and 92% after the 2011 guideline published jointly by the American Thoracic Society, the European Respiratory Society, and the International Association for the Study of Lung Cancer. They performed both EGFR and ALK testing, and it is unclear what changed between the two time periods. Arcila et al. were able to perform EGFR sequencing on 89% and 79% on their CNB and FNA specimens, respectively. Even if we included our seven samples for which EGFR but not ALK testing worked (success rate of 52%), their yield is still much higher despite their sampling of treatment-resistant cancers in which one might expect a lower success rate due to treatment-related changes.

Solomon et al. report that 89% of their CNB specimens were adequate for EGFR and KRAS sequencing. In all of these studies, it is unclear whether the denominator of the success rate comprises all adenocarcinomas eligible for testing or only the cases on which testing was attempted. For example, report a 91% success rate for EGFR and ALK testing on cytology specimens but specifically include only those cases on which such testing was requested. When all cases are considered, such as by Hsiao et al. who obtained EGFR results on 132 (80%) of a total of 164 CNB specimens, success rates are lower and similar to ours. More in keeping with our data is the experience by Knoepf and Roh, who report that 37% of FNA cell block preparations were acellular and another 20% sparse or borderline. Khan et al. found that 25% of their FNA specimens did not have enough cells for immunohistochemical stains, likely an indicator that at least as many cases would also be insufficient for molecular studies. Each practice has to decide whether reflex testing on small biopsy samples is desirable or useful.

Here, this practice pattern allowed us to establish success rates of CNBs and FNAs.

In our study, the mean number of FNA passes for sufficient biopsy specimens was higher than that for insufficient FNA specimens, although this difference was not statistically significant. Intuitively, a higher number of FNA passes should be more likely to yield a sample suitable for molecular testing. However, our results and published data show that an increased number of passes does not necessarily result in a higher adequacy rate. Instead, performing immediate on-site assessments may be the best way to ensure adequacy of the sample. Counting the number of biopsy passes before and after implementing our new policy to request two additional FNA passes solely for cell block preparation helped assess implementation. Although the mean number of passes did not increase by two, a tendency to obtain more tissue was apparent. Our biopsy operators do not employ coaxial biopsy needle systems for FNA specimens, so there may be a reluctance to perform two additional punctures for fear of complications once diagnostic material has been obtained. Some of this concern may be alleviated by data showing that neither the number of needle passes nor the dwell time of a coaxial needle are associated with an increased incidence of pneumothorax. We also observed a reluctance of operators to perform CNBs, usually due to...
the risk of creating a pneumothorax.20 The true risk of pneumothorax may be difficult to estimate for operators, and use of smaller core needles can reduce that risk.21,22

Our comparison shows that there are significant differences between operators and/or biopsy technique. With some FNA operators, patients had a greater than 50% chance of requiring a repeat biopsy if molecular testing was needed. Published data on operator variability in image-guided biopsies are essentially nonexistent, although they are likely subject to confidential departmental quality assurance. Our numbers have to be interpreted with caution since operator performance is influenced by numerous variables, including patient characteristics, location and depth of the targeted lesion, fidelity and imaging speed of the CT scanner, and diameter and tip design of the biopsy needles. Most important, our comparison shows that even the most skilled FNA operator did not reach the adequacy rate of CNBs.

We acknowledge several limitations of this study. Our institution prefers to perform molecular testing on cytology specimens through a similar platform used for our surgical pathology samples. Therefore, we are only comparing cell block preparations from FNA material with paraffin-embedded CNB specimens. Cell block preparations can be cell poor despite adequately cellular aspirates, and higher success rates could possibly be achieved by scraping tumor cells directly from cytology smears.47 However, we prefer to leave cytology slides intact, especially if tumor cells are scant. Pathology practices submitting their tissue samples to referral laboratories for testing may also be less inclined to give up their original diagnostic cytology smears. Introducing ALK immunohistochemistry as a surrogate marker for ALK rearrangement could possibly lower the rate of insufficient ALK FISH samples.23 We also did not control for patient age, biopsy risk factors, or location of the targeted mass. We suspect that such variations were evenly distributed and differences negligible in our study population that included consecutive biopsy specimens diagnosed as adenocarcinoma or non–small cell carcinoma, favor adenocarcinoma. However, we cannot exclude that patients who underwent CNBs were “easier to biopsy.” We suspect that intratumor variability of oncogenic mutations may not be very common as initial reports suggested. Recent studies confirmed homogeneity of driver mutations in primary and metastatic lung carcinomas. For example, Yatabe et al24 suggested the concept of pseudoheterogeneity of EGFR mutations. They found identical EGFR driver mutations in multiple areas of the same tumor. However, the presence of EGFR amplification and/or contamination by normal cells may interfere with the interpretation of PCR mutation assays.24 In our setting, the main issue would be the detection limit when mutated tumor cells are admixed with nonmutated tumor cells. Taniguchi et al25 showed that EGFR mutations can still be detected in areas that contain nonmutated cells. Vignot et al26 were able to identify the driver mutation in lung cancer even if a tumor had accumulated other molecular alterations.

Different pathologists could potentially influence the success rate of FNAs by not requesting testing on cell blocks that contain sufficient material. This seemed unlikely because all pathologists were educated on the minimum number of cells required by the laboratory. Also, since pathologists are aware of the importance of molecular testing, they tend to err on the side of caution and are more likely to request testing on cell blocks or CNB specimens that later turn out to be insufficient. Even if our minimum number of cells were too high, it should not influence the direct comparison between CNB and FNA specimens in this study.
The present study is unique because during the study period, we performed reflex molecular testing on eligible patients with lung carcinoma. Therefore, we can use cases that were eligible for testing but did not contain sufficient material for calculation of our adequacy rate. Most studies reporting high success rates of molecular testing do not consider in their calculation of success rates those biopsy specimens on which testing was not even attempted. Furthermore, to our knowledge, this is the only study to date that compares directly the yield of CNB and FNA samples for EGFR, KRAS, and ALK testing in the same clinical environment. Although the 67% success rate of CNBs still leaves room for improvement, not being able to offer patients molecular testing on more than half of the FNA specimens is undesirable. For clinical environments in which only paraffin-embedded tissue is used for molecular testing of lung cancer, our data suggest that (1) CNBs are preferable over FNAs to obtain adequate tissue for molecular testing of lung adenocarcinomas, and (2) FNAs are more likely to yield sufficient samples from nodules above a certain size.

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References


