ORIGINAL ARTICLE

Percutaneous Image-Guided Biopsy for a Comprehensive Hybridization Capture-Based Next-Generation Sequencing in Primary Lung Cancer: Safety, Efficacy, and Predictors of Outcome

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ABSTRACT

Introduction: To evaluate factors associated with successful comprehensive genomic sequencing of image-guided percutaneous needle biopsies in patients with lung cancer using a broad hybrid capture-based next-generation sequencing assay (CHCA).

Methods: We conducted a single-institution retrospective review of image-guided percutaneous transthoracic needle biopsies from January 2018 to December 2019. Samples with confirmed diagnosis of primary lung cancer and for which CHCA had been attempted were identified. Pathologic, clinical data and results of the CHCA were reviewed. Covariates associated with CHCA success were tested for using Fisher's exact test or Wilcoxon ranked sum test. Logistic regression was used to identify factors independently associated with likelihood of CHCA success.

Results: CHCA was requested for 479 samples and was successful for 433 (91%), with a median coverage depth of 659X. Factors independently associated with lower likelihood of CHCA success included small tumor size (OR = 0.26 [95% confidence interval (CI): 0.11–0.62, $p = 0.002$]), intraoperative inadequacy on cytologic assessment (OR = 0.18 [95% CI: 0.06–0.63, $p = 0.005$]), small caliber needles (≥20-gauge) (OR = 0.22 [95% CI: 0.10–0.45, $p < 0.001$]), and presence of lung parenchymal abnormalities (OR = 0.12 [95% CI: 0.05–0.25, $p < 0.001$]). Pneumothorax requiring chest tube insertion occurred in 6% of the procedures. No grade IV complications or procedure-related deaths were reported.

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Conclusions: Percutaneous image-guided transthoracic needle biopsy is safe and has 91% success rate for CHCA in primary lung cancer. Intraoperative inadequacy, small caliber needle, presence of parenchymal abnormalities, and small tumor size ($\leq$1 cm) are independently associated with likelihood of failure.

Keywords: Comprehensive hybridization capture-based next-generation sequencing; Gene assay; Lung cancer; Percutaneous transthoracic biopsy

Introduction

Lung cancer is the second most common cancer in the United States and the leading cause of cancer death.[1] Efforts to improve response to this devastating disease have focused on driver genomic alterations. Therapies targeting these drivers are considered standard of care and cited in practice guidelines for lung cancer management.[2–6]

The use of broad, hybrid capture-based next-generation sequencing (NGS) assays compared with polymerase chain reaction (PCR)-based assays has been highlighted in recent guidelines to increase sensitivity of detecting copy number alterations and gene fusions, quantify tumor mutation burden, improve efficiency of patient matching for clinical trials, and disambiguate primary lung carcinomas from intrapulmonary metastases.[7–10] In recent years, our comprehensive hybrid capture-based NGS assay (CHCA), was cleared by the U.S. Food and Drug Administration as an in vitro test for solid cancer. Using this assay, 37.1% of patients with lung cancer received matched treatment, with reported clinical benefit of 85.8% and 76% in level 1 and level 2A alterations, respectively.[11]

Nevertheless, such assays may require higher quantities of DNA from high-quality biopsy samples than amplicon-based or PCR-based assays and other traditional molecular tests which query a very limited number of genes.[12] Considerations for sufficient tumor proportion on the biopsy are also of critical importance.

Thus, image-guided biopsy in lung cancer has shifted from obtaining specimens that are only sufficient for histopathologic diagnosis to acquiring larger amounts of viable tumor cells that contain a high quality and quantity of DNA for NGS. Several studies have confirmed the feasibility and safety of using percutaneous transthoracic core needle biopsy for conventional molecular assays, such as *EGFR*, *ALK*, and *KRAS* testing in lung cancer.[13–16] There are some reports that evaluate the feasibility of using core needle biopsy specimens for NGS.[17–23] Nevertheless, these reports primarily evaluate feasibility using small multigene PCR- or amplicon-based NGS panels and limited reports using large hybrid capture-based NGS panels that test for hundreds of genes, such as the CHCA.[19–21] Our group has previously published on the feasibility of testing small cytologic and biopsy samples and the protocols associated with fine-tuning the processing and extraction procedures to maximize the final yield of material available for molecular testing.[24–26] But a dedicated analysis of the pre-analytical variables associated with obtaining the biopsies has not been performed.

Therefore, our study aims to evaluate safety and efficacy and to identify factors associated with successful genomic analysis of image-guided percutaneous needle biopsy in patients with primary lung carcinoma performed by multiple operators and tested using a CHCA.

Materials and Methods

Patients

We conducted a single-institution retrospective review of patients who underwent image-guided percutaneous transthoracic needle biopsy from January 2018 to December 2019. A search of our hospital electronic medical records yielded a total of 2781 lung biopsies. All biopsies with confirmed diagnosis of primary lung cancer that included a CHCA request sent to our diagnostic molecular pathology laboratory were included in the study. After thorough review with the diagnostic molecular pathology department, CHCA requests processed from out-of-hospital or referred specimens, surgical specimens, and endoscopy specimens were excluded. Finally, 479 biopsies were included in our analysis. The study was compliant with the Health Insurance Portability and Accountability Act and was approved by the Institutional Review Board with a waiver of informed consent.

Data Collection and Analysis

The medical records of all patients were reviewed for the following parameters: demographics; previous treatments; histopathologic assessment; imaging guidance; imaging reference; lesion size and location; and procedural details, including needle size, imaging findings, procedural complications, and success rate of the assay. Histologic assessment was based on the 2015 WHO Classification of Lung Tumors.[27] Two board-certified radiologists reviewed preprocedure and procedure imaging independently and blinded to the results of the assay. Lesion size was recorded as maximum diameter on computed tomography (CT) images with

standard lung window settings. Lesion distribution in the lung field was defined as central or peripheral. The central lesion includes any hilar or mediastinal lesion adjacent to a central airway, the heart, or a major vascular structure, such as the main pulmonary artery or thoracic aorta.[28] Lesion type was categorized as pure solid, subsolid, or pure ground-glass opacity.[29] Additional morphologic characteristics were defined as presence of necrosis (low density area within the lesion) or cavitation (air-filled cavity within the lung lesion) on the basis of imaging evaluation.[29] Surrounding abnormal lung parenchyma included presence of any underlying/surrounding consolidation (areas of parenchymal opacification obscuring underlying lung markings with or without air bronchograms), fibrosis (fine or coarse reticulations, thick nodular opacities ± thickening of interlobular/interlobar septa ± bronchiectasis), or atelectasis (increased local density of the atelectatic portion ± displacement of interlobar fissures ± crowdedness of pulmonary vessels or air bronchograms).

Samples submitted for CHCA were examined, and success was defined as the ability to perform CHCA using DNA extracted from the specimen and the presence of sufficient tumor (>10%) to enable detection of variants. Samples that produced an unsuccessful assay were reviewed pathologically to identify reasons for failure and to evaluate whether any other molecular profiling tests were alternatively performed from these samples. The reasons for CHCA failure were categorized as follows: (1) nondiagnostic sample (sampling error), (2) unprocessed for DNA extraction due to insufficient tissue volume or low tumor content, (3) low DNA yield (<50 ng), and (4) technical failure.

Biopsy Technique

All biopsies were performed by or under the supervision of a board-certified interventional radiology (IR) specialist. Anticoagulation and antiplatelet medications were held, and coagulopathy was corrected before the procedure per consensus guidelines.[30] The operators were always aware of the indication of the biopsy. These were either for "primary diagnosis and follow-up" or for "molecular" analysis; however, the plans for CHCA were not always available to the IR physician at the time of lesion selection or biopsy. An attending IR physician reviewed available reference diagnostic imaging. Procedure was performed under moderate sedation. The most often used imaging guidance modalities were CT and CT fluoroscopy. In very few cases, positron emission tomography (PET)/CT or ultrasound was used. The imaging guidance modality of choice largely depended on the IR physician's preference or experience, the target location and depth in the parenchyma, and the accessibility of the technical devices. All biopsies were performed using a coaxial needle system (Mission Disposable Core Biopsy Instrument, BD, Franklin Lakes, NJ; Bard Mission Westcott Needle, Bard Biopsy, Tempe, AZ; Temno Evolution or Adjustable Coaxial Temno, CareFusion, BD, Franklin Lakes, NJ) with an 18- or 20-gauge (G) core biopsy needle for core biopsy and 20-G or 22-G fine-needle aspiration (FNA) needle. A cytopathologic technologist was present for all biopsies, and the touch preparation technique was used for immediate inspection of sample adequacy. The number of core or FNA specimens was determined according to the individual operator's discretion, findings at on-site cytologic examination, size of the specimens obtained, and special requirements if the biopsy was requested for a research protocol. The choice of biopsy needle gauge was determined by the operator's preference. Targeted tumor size, number of specimens taken, biopsy device, and needle gauge used were recorded. Complications of needle biopsy such as pneumothorax requiring a chest tube were noted following the Society of Interventional Radiology definition and grading system.[31] After biopsy, inspiratory chest radiographs with the patient in an erect position were obtained immediately and at two hours postbiopsy. A chest tube was inserted if the pneumothorax size was large (>30% of lung volume); if the pneumothorax increased in size; or if the patient experienced pain, dyspnea, or a decrease in oxygen saturation. Patients who had chest tubes placed were treated as outpatients or were admitted to the hospital for management.

Specimen Processing and Molecular Diagnostics Analysis

On-site cytopathology assessment was available in all procedures for preliminary evaluation of sample adequacy. Specimens were fixed in neutral-buffered formalin and embedded in paraffin. Tissue sections of 5 μm in thickness were prepared. For all cases, 15 to 20 recuts were prepared for DNA extraction and at least one hematoxylin and eosin–stained section was reviewed to determine whether there was at least 10% tumor content in the specimen. Cases were rejected if they contained less than 10% of the tumor content. Sequencing of matched tumor and blood DNA was performed using the clinically validated CHCA.

DNA was extracted using the DNeasy Tissue KIT (Qiagen, Valencia, CA). Quantification was performed using 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 less comprehensive NGS panels. Specimens that were successful for CHCA and met the

assay performance criteria (>10% proportion of tumor cells by visual estimate and >minimum of 50 ng input DNA) but in which tumor cells represented less than 20% of the total nucleated cells within the sample were flagged as having relatively low tumor content. Captured DNA fragments were sequenced on an Illumina HiSeq2500 system (Illumina, San Diego, CA) before bioinformatics analysis pipeline. In this assay, matched normal DNA from blood is simultaneously processed with tumor DNA for all samples, allowing identification and filtering of germline variants.

All samples were processed with the same CHCA, which analyzes 468 cancer-related genes. Mutations were reported only if the variant calling reached a frequency of 2% for the clinically validated panel in 19 genes and 5% for all other genes in the investigational panel. Tumor samples were required to be sequenced to at least 200× coverage. Parameters of tumor and DNA quantification and assay performance were reviewed for all. 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, structural variants, tumor mutational burden, microsatellite instability (MSI) score, and status. In addition, the rate of mitogenic driver detection and the recommended targeted therapy for the detected mutations were determined.

Statistical Analysis

Continuous and categorical data were presented as median with interquartile range (IQR) and as number and percentage, respectively. Distribution of clinical and procedural characteristics for successful versus unsuccessful CHCA was compared using Fisher's exact test or the Wilcoxon ranked sum test for categorical and continuous variables, respectively.

Logistic regression models were used to identify factors independently associated with likelihood of CHCA success. Factors that differed significantly ($p < 0.05$) and were considered clinically relevant (including lesion size, adequacy in procedure room, needle size, sample length, presence of abnormal lung parenchyma, prior radiation, and systemic therapy) were selected for multivariable analysis. A natural log transformation was applied to the sample length due to the skewed distribution. Lesion size was analyzed as a binary variable, dichotomized at cut point less than or equal to 1.0 versus more than 1.0 cm on the basis of previous studies. Owing to the limited sample size of patients with unsuccessful CHCA, a backward selection approach was used to trim down to a parsimonious model retaining only variables with p value less than 0.05. ORs with 95% confidence intervals (CIs) were estimated for univariable and multivariable models to quantify the magnitude and directionality of association. A p value less than 0.05 was considered statistically significant. All statistical analyses were performed with software R version 3.6.1 (The R Foundation for Statistical Computing, Vienna, Austria).

Results

Patients and Procedure Variables

This study included 479 biopsies performed on 462 patients in two consecutive years (Fig. 1) by 26 interventional radiologists and sent for CHCA; 446 patients (96.5%) had one biopsy, 15 patients (3.2%) had two biopsies, and one patient (0.2%) had three biopsies.

Overall, 265 patients (57%) were female and 197 (43%) were male. The median (IQR) age of all 479 patients was 69 (62–76) years. Furthermore, 478 biopsies were adequate for making the histologic diagnosis of primary lung cancer. One biopsy taken from a patient with active lung adenocarcinoma under treatment and sent for CHCA was not sufficient to make histologic diagnosis. Nevertheless, CHCA was performed successfully from a subsequent image-guided biopsy taken from a different lesion in that patient.

The median size of the biopsied lesions was 2.7 cm (IQR = 1.7–4.2). Approximately 11% (52 of 479) of the biopsied lesions were less than or equal to 1 cm. The most common histologic type was adenocarcinoma (75%). In addition, 289 biopsies (60%) were taken from patients with no history of prior or ongoing systemic therapy and 428 biopsies (89%) had no prior history of radiation. Conventional CT guidance was most often used (89%). Other imaging guided modalities used included CT fluoroscopy (8.4%), ultrasound (2.1%), and PET/CT (0.4%). The 18-G needles were used in 56% of biopsies, whereas more than or equal to 20-G were used in 44%. Median and IQR of number of samples taken were three (3–4). Other variables are summarized in Tables 1 and 2.

Feasibility and Predictors of Success

The success rate for CHCA was 91% (433 of 479). Reasons of the 9% (46 of 479) failure were as follows: (1) nondiagnostic sample (1 of 46 = 2%); (2) unprocessed for DNA extraction due to insufficient tissue volume (10 of 46 = 22%) or low tumor content (13 of 46 = 28%); (3) low DNA yield (<0.1 ng/μL) (20 of 46 = 44%); and (4) technical failure due to low coverage

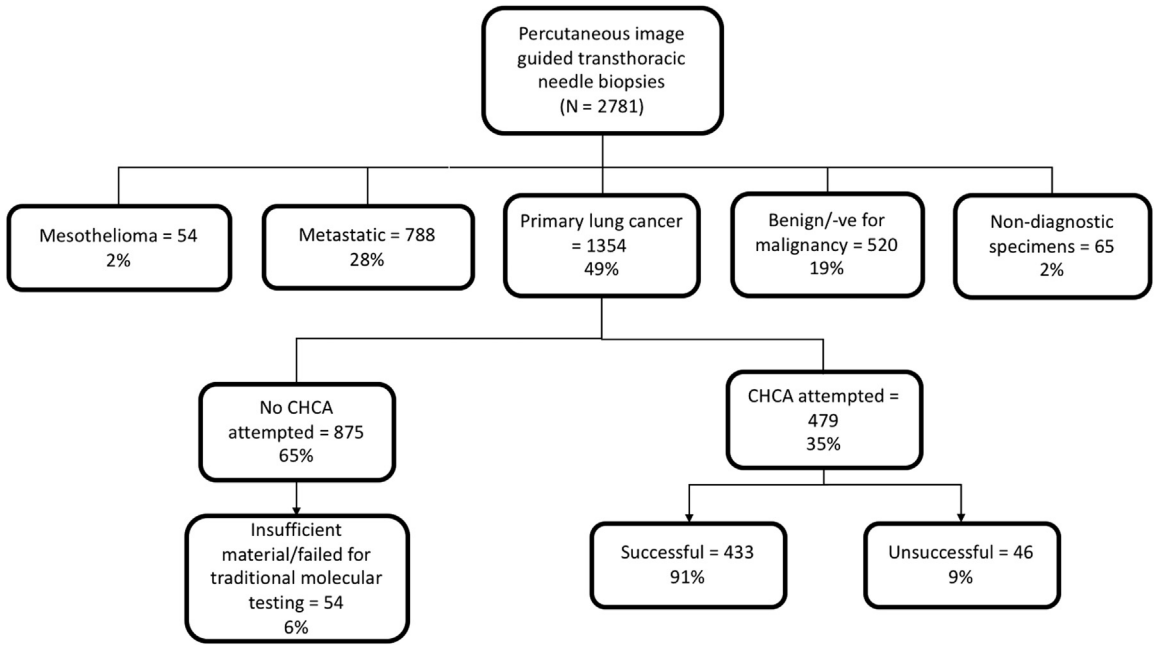

Figure 1. Flowchart of percutaneous image-guided transthoracic needle procedures performed during the study period. Numbers refer to individual image-guided procedures. Numbers refer to individual image-guided procedures. CHCA, comprehensive hybridization captured-based next-generation sequencing assay.

(<200×) (2 of 46 = 4%). Of the 46 samples that were unsuccessful in CHCA testing, 19 (41.3%) underwent molecular testing by an alternative method. In total, 92% (441 of 479) and 94.4% (452 of 479) were successful in some form of NGS or molecular testing, respectively. CHCA was performed successfully from subsequent image-guided biopsies of lung lesions in three unsuccessful samples.

Compared with successful biopsies, unsuccessful biopsies had significantly higher rates of smaller target sizes (≤1 cm) ($p = 0.01$), small caliber needles (≥20-G) ($p < 0.001$), FNA biopsies ($p = 0.007$), abnormal surrounding lung parenchyma ($p < 0.001$), prior radiation ($p = 0.02$), and prior/ongoing systemic therapy ($p = 0.039$). The median sample length was significantly larger in the successful samples (0.8 versus 1 cm, $p = 0.032$). Unsuccessful biopsies were associated with significantly higher rates of inadequacy on intraoperative cytopathologic assessment ($p = 0.004$). Higher rates of postbiopsy changes in the form of perilesional hemorrhage were more frequently detected in unsuccessful biopsies, although the difference was not statistically significant ($p = 0.052$). The median number of samples taken and central location of lesion were not statistically different between successful and unsuccessful biopsies.

In multivariate logistic regression analysis (Table 3), factors independently associated with lower likelihood of CHCA success included small tumor size (≤1 cm) (OR = 0.26 [95% CI: 0.11–0.62, $p = 0.002$]), inadequacy on procedure room cytologic assessment (OR = 0.18 [95% CI: 0.06–0.63, $p = 0.005$]), use of small caliber needles (≥20-G) (OR = 0.22 [95% CI: 0.10–0.45, $p < 0.001$]), and presence of abnormal surrounding lung parenchyma (OR = 0.12 [95% CI: 0.05–0.25, $p < 0.001$]). Sample length and presence of prior radiation and systemic therapy did not retain significance on multivariate analysis. Success rate in more than 1-cm core biopsies was 92.6% (387 of 418).

CHCA Analysis Outcome

The assay was completed successfully on 433 of the 497 biopsy samples (91%), identifying a median (IQR) of eight (5–14) mutations at a median coverage depth of 659X. Most of these samples (370 [85.5%]) had at least one mutation detected in the clinically validated panel. Median (IQR) MSI score was 0.05 (0–0.3); MSI status was MSI-stable in most of the samples (96%), MSI-indeterminate in 1.9% of the samples, and MSI-high in 1.7% of the samples. Median and IQR of tumor mutational burden (total number of nonsynonymous somatic mutations identified per megabase) in all the samples was 5.3 m per megabase (2.6–8.8). Tumor purity (% of cancer cells in the sample) was less than 20 in 23%, 20 to 40 in 64%, and more than 40 in 13% of the samples. Specimen quantity and quality were judged to be fully adequate in 335 of the 433 successful samples (77.4%). In 98 samples (22.6%), the CHCA was completed

Table 1. Demographics and Clinical Characteristics

Characteristics	All (N = 479)[a]	Success (n = 433)[a]	Failure (n = 46)[a]	*p* Value[b]
Age	69 (62-76)	69 (62-76)	67 (59-75)	0.2
Sex				0.9
F	277 (58)	251 (58)	26 (57)	
M	202 (42)	182 (42)	20 (43)	
Histologic type				0.6
Adenocarcinoma	358 (75)	327 (76)	31 (69)	
Sq. cell carcinoma	69 (14)	60 (14)	9 (20)	
NSCLC-NOS	14 (2.9)	12 (2.8)	2 (4.4)	
Sarcomatoid	6 (1.3)	6 (1.4)	0 (0)	
Pulmonary neuroendocrine tumors	31 (6.5)	28 (6.5)	3 (6.7)	
Nondiagnostic	1	—	1	
Tumor size				**0.01**
≤1 cm	52 (11)	41 (9.5)	11 (24)	
>1 cm	427 (89)	392 (91)	35 (76)	
Pleural/subpleural				0.9
No	239 (50)	217 (50)	22 (48)	
Yes	240 (50)	216 (50)	24 (52)	
Distribution				0.06
Central	139 (29)	120 (28)	19 (41)	
Peripheral	340 (71)	313 (72)	27 (59)	
Additional morphologic characteristics				0.2
No	435 (91)	396 (91)	39 (85)	
Yes	44 (9.2)	37 (8.5)	7 (15)	
Lesion type				0.3
Solid	417 (87)	379 (88)	38 (83)	
Subsolid	41 (8.6)	37 (8.5)	4 (8.7)	
GGO	21 (4.4)	17 (3.9)	4 (8.7)	
Abnormal surrounding lung parenchyma				**<0.001**
No	403 (84)	378 (87)	25 (54)	
Yes	76 (16)	55 (13)	21 (46)	
Prior radiation				**0.020**
No	428 (89)	392 (91)	36 (78)	
Yes	51 (11)	41 (9.5)	10 (22)	
Prior/ongoing systemic therapy				**0.039**
No	289 (60)	268 (62)	21 (46)	
Yes	190 (40)	165 (38)	25 (54)	
Prior resection				0.7
No	406 (85)	368 (85)	38 (83)	
Yes	73 (15)	65 (15)	8 (17)	

Note. Bold emphasis is for $p < 0.05$.
[a]Median (IQR); n (%).
[b]Wilcoxon ranked sum test; Fisher's exact test.
F, female; GGO, ground-glass opacity; IQR, interquartile range; M, male; NSCLC-NOS, NSCLC, not otherwise specified.

successfully but the samples were found to have relatively low tumor content (<20%) which could interfere with detection of copy number alterations, fusions, and detection of the full spectrum of mutations.

Complications

Adverse events associated with the biopsy procedure were graded as per Society of Interventional Radiology guidelines and included 6.1% (29 of 479) pneumothorax requiring chest tube insertion (Table 4). Four patients who underwent chest tube insertion were discharged on the day of the procedure after chest tube removal, whereas 25 were admitted. Median (IQR) hospital stay among admitted patients was 1 day (0–2). All chest tubes were removed before discharge. Postprocedure effusion was detected in six procedures (1.2%), of which five were grade I with no intervention needed, and only one was completely controlled after ultrasound-guided thoracocentesis (grade II). Grade I hemoptysis was recorded in 16 of 479 (3.3%) procedures. No grade IV complications or procedure-related deaths were reported.

Compared with small caliber needles (≥20-G), use of large caliber needles (18-G) was not significantly associated with higher rates of pneumothorax requiring chest tube (4.9% in ≤18-G versus 7.6% in ≥20-G, $p =$

Table 2. Procedure Characteristics

Characteristics	All (N = 479)[a]	Success (n = 433)[a]	Failure (n = 46)[a]	*p* Value[b]
In-room cytology				**0.004**
Adequate	459 (96)	419 (97)	40 (87)	
Inadequate/adequacy not confirmed	18 (3.8)	12 (2.8)	6 (13)	
NA	2	2	—	
Imaging guidance				0.8
CT	426 (89)	386 (89)	41 (89)	
CT and CT fluoroscopy	40 (8.4)	36 (8.3)	4 (8.7)	
US	10 (2.1)	9 (2.1)	1 (2.2)	
PET/CT	2 (0.4)	2 (0.5)	0 (0)	
Imaging reference				0.3
CT	288 (60)	257 (59)	31 (67)	
PET/CT	191 (40)	176 (41)	15 (33)	
Needle size				**<0.001**
18 G	268 (56)	255 (59)	13 (28)	
≥20 G	211 (44)	178 (41)	33 (72)	
Number of samples	3 (3-4)	3 (3-4)	3 (3-5)	0.7
NA	14	14	-	
Sample length (cm)	1 (0.70-1.40)	1 (0.70-1.40)	0.8 (0.40-1.17)	**0.032**
NA	12	8	4	
Sample type				**0.007**
Core	470 (98)	428 (99)	42 (91)	
FNA	9 (1.9)	5 (1.2)	4 (8.7)	
Indication				0.3
Primary diagnosis/follow-up	298 (62)	273 (63)	25 (54)	
Molecular testing/research protocol	181 (38)	160 (3%)	21 (46)	
Number of interventional radiologists	26	26	17	0.4

Note. Bold emphasis is for $p < 0.05$.
[a]Median (IQR); n (%).
[b]Wilcoxon ranked sum test; Fisher's exact test.
CT, computed tomography; FNA, fine-needle aspiration; G, gauge; IQR, interquartile range; NA, not available; PET, positron emission tomography; US, ultrasound.

0.2) or hemoptysis (3.7% in ≤18-G versus 2.8% in ≥20-G, $p = 0.8$). The median number of samples/cores was not significantly different between the biopsy-related pneumothorax group and the nonpneumothorax group (three cores for both groups, $p = 0.7$) or between patients who required chest tube insertion and patients who did not (three cores for both groups, $p = 0.4$). Biopsies with concurrent hemoptysis had a significantly lower median number of samples/cores than biopsies with no concurrent hemoptysis (two versus three samples, $p < 0.001$).

Discussion

This study reveals the high success rate (91%) of percutaneous image-guided biopsy for CHCA performed by 26 interventional radiologists. We recorded several factors associated with the outcome of the percutaneous biopsy specimens used in the CHCA.

The reported success rate of the use of NGS in percutaneous image-guided biopsy specimens in large cohort studies ranges from 69.9% to 95.3%.[17,18,20,32,33] Nevertheless, these reports include NGS from multiple locations (not just lung) and primarily used small multigene NGS panels (<150 genes). In this study, we evaluate the feasibility of percutaneous image-guided biopsies for a large hybridization capture-based NGS panel that tests 468 cancer-related genes in a large cohort of patients with primary lung cancer. We also analyzed patient-, procedure-, and target lesion-specific factors to identify independent factors for successful genomic analysis.

Previous studies have suggested that small lesion size (<1 cm) obtained by percutaneous needle biopsy is associated with higher rates of insufficient DNA isolation.[34] In our study, lesions less than or equal to 1 cm in maximal diameter had lower odds of success in CHCA than lesions more than 1 cm on multivariate analysis. Nonetheless, in lesions less than or equal to 1 cm, we were able to obtain a satisfactory sample for CHCA in 79% of the cases and the success rate for the lesions more than 1 cm was 92% (392 of 427).

The presence of viable tumor tissue (rather than inflammation, stroma, and post-treatment fibrosis) and favorable surrounding tissue are important factors for obtaining higher DNA yield from the target lesion.[35] Our study revealed that the presence of abnormal surrounding lung parenchyma (i.e., consolidation,

Table 3. Multivariate Logistic Regression Model of Variables Associated With Success for Comprehensive Hybridization Capture-Based Assay

	Multivariate Analysis	
Variables	OR (95% CI)	*p* Value
Tumor size		
>1 cm	1.00	
≤1 cm	0.26 (0.11-0.62)	**0.002**
In-room cytology		
Adequate	1.00	
Inadequate/adequacy not confirmed	0.18 (0.06-0.63)	**0.005**
Needle size		
18-G	1.00	
≥20-G	0.22 (0.10-0.45)	**<0.001**
Sample length (log transformed)	–	–
Abnormal surrounding lung parenchyma		
No		
Yes	0.12 (0.05, 0.25)	**<0.001**
Prior radiation		
No	–	
Yes	–	
Prior/ongoing systemic therapy		
No	–	
Yes	–	

Note. All variables significant on univariate analysis that were included as initial input to the backward selection method are found. Variables that were removed during backward selection do not have associated OR or *p* value. Bold emphasis is for $p < 0.05$.
CI, confidence interval.

atelectasis, or fibrosis) was associated with lower likelihood of success on multivariate analysis. Use of real-time ^{18}F-fluorodeoxyglucose-PET/CT as imaging guidance could improve target localization and allow physicians to choose the best site with viable tumor tissue for biopsy, especially in the presence of abnormal surrounding lung parenchyma or necrosis. Nevertheless, in our study, real-time intraprocedural ^{18}F-fluorodeoxyglucose-PET/CT was used in only two procedures.

The choice of needle size and number of cores have been associated with the DNA yield acquired by percutaneous needle biopsy for NGS. A prospective study comparing 18-G and 20-G core needles for lung nodules revealed that, to optimize yield, a lower gauge needle with a single pass is preferable to a higher gauge needle with two passes.[36] Another study concluded that the use of 20-G side-cut core biopsy needles requires a increased number of passes to ensure diagnostic adequacy for molecular testing across all tissue types, and the use of 16-G or 18-G needles markedly reduces the number of passes required to obtain similar yields.[37] Multivariate analysis revealed that the use of small caliber needles (≥20-G) had lower likelihood of CHCA success than large caliber needles (18-G). The median (IQR) number of cores was not statistically different between successful (three samples, 3–4) and unsuccessful biopsies (three samples, 3–5). In the current study, large caliber needles (18-G) were not significantly associated with higher rates of pneumothorax requiring chest tube or hemoptysis compared with small caliber needles (≥20 G). Therefore, use of an 18-G needle in the appropriate clinical setting was found to increase the success rate for CHCA without significant increase in the complication rate compared with the use of a 20-G needle.

Intraoperative cytopathologic evaluation has been found to improve diagnostic accuracy and yield for molecular diagnostics and to reduce needle passes in image-guided transthoracic needle aspiration.[35,38] At our institution, intraprocedural cytopathologic evaluation is implemented routinely in all percutaneous-guided needle biopsy. Inadequacy on intraoperative cytologic assessment was associated with lower likelihood of CHCA success compared with adequate samples ($p = 0.005$).

The importance of tumor heterogeneity in lung cancer is well established both in the context of histologic subtypes[39,40] and in the molecular heterogeneity and subclonal architecture of the tumors.[41] We have previously reported on the accuracy and sensitivity of percutaneous core biopsy in identifying heterogeneous histologic subtypes of lung adenocarcinoma using appropriate needle gauge size and number of cores.[42] The relationship between histologic subtype heterogeneity and molecular heterogeneity was recently elucidated by Tang et al.,[43] who found that transcriptomic profiles but NOT genomic profiles were associated with histologic subtype.

Table 4. Adverse Events Using SIR Grading System

	I	II	III	IV	V	
Complication	Mild: Nominal Therapy, Observation	Moderate: Substantial Intervention or Overnight Hospitalization	Severe: Major Therapy, Prolonged Hospitalization	Life-Threatening: Permanent Adverse Sequelae	Death	Total
Pneumothorax	4	25	0	0	0	29
Pleural effusion	5	1	0	0	0	6
Hemoptysis	16	0	0	0	0	16
Total	25	26	0	0	0	51

SIR, Society of Interventional Radiology.

In this regard, heterogeneity of histologic subtype is likely not as relevant to the CHCA results. Markers of genomic heterogeneity including tumor mutation burden have been associated with response to immunotherapy in some settings.[44] The ability of needle core biopsy to capture genomic heterogeneity and additional immunotherapy predictive biomarkers is a focus for future work.

The role of endoscopic biopsy and endobronchial ultrasound transbronchial needle aspiration has been described in previous studies of NGS in lung cancer.[45,46] Needle gauge size was smaller with endoscopic approaches. A recent study reported an endoscopic biopsy and endobronchial ultrasound transbronchial needle aspiration success rate of 86% for the same CHCA.[12] Although it excluded nondiagnostic samples, the success rate based on general biopsy attempt is unknown. This success rate can be highly variable depending on the location of the lesion, the accessibility, and the individual performing the procedure. In our study, 50% of the lesions were located in a pleural or subpleural location; 28% were located centrally in the hilum, around the cardiac border, or around large vascular structures, such as the main pulmonary artery or thoracic aorta. Although our analysis included nondiagnostic specimens for which CHCA analysis was requested, similar to other studies, the overall failure rate remains unknown as some of the unsuitable samples would not have had molecular testing requested.

The overall complication rate of percutaneous-guided biopsy for NGS is comparable with that of conventional biopsy performed for histologic diagnosis.[20] The reported chest tube rates after transthoracic image-guided biopsy are in the range of 1.6% to 15% for drainage catheter insertion.[14,47] In our study, 6.1% of the patients required chest tube insertion.

This study has several limitations. This retrospective study represents data from a single institution, which is a well-established cancer center with the requisite infrastructure and experience and a multidisciplinary team consisting of interventional radiologists, medical oncologists, surgeons, cytopathologists, and molecular pathologists. The biopsy procedures were performed by IRs with a variable degree of experience ranging from 5 to 25 years. The operating IR physician is often aware of the indication of the biopsy before performing the procedure, which can be either "primary diagnosis and follow-up" or "molecular." Nevertheless, in some cases, plans to involve CHCA were not released. This could lead to bias as the number of cores and needle size may be influenced by the indication and plan to perform CHCA. Notably, we did not see any association between indication and CHCA success. Choice of needle size, imaging modality, and number of cores are largely dependent on each operator's preference and expertise. NGS panels differ in sensitivity and specificity on the basis of the minimum quantity and quality of DNA required to run the test and the technology involved for genomic analysis. Our study used a well-established CHCA and all tissue handling, and extraction procedures have been finetuned to maximize the yield and performance for this assay. Of note, in this study, we did not include a large number of patients for which biopsies were performed but request for CHCA testing was not submitted. This could potentially lead to bias, either due to knowledge that specimens were of poorer quality in general, or adequate samples that did not require comprehensive assessment due to lack of the required consent or a positive result by a prior assay. At our institution, we offer rapid testing by single gene assays, such as *EGFR*, before NGS testing. Although most cases go on to be tested by CHCA regardless of result, a proportion of positive cases may not be further tested. Of the 65% (875 of 1354) of the primary lung cancer biopsies that were not submitted for CHCA testing, we identified only 6% (54 of 875) that were reported to have insufficient material for further molecular testing or failed traditional molecular tests due to inadequate material.

In conclusion, percutaneous image-guided transthoracic needle biopsy is safe and has a high success rate for comprehensive, hybrid capture-based large gene NGS

panels in primary lung cancer. Inadequacy on intraoperative cytologic assessment, use of small caliber needle ($\geq$20-G), presence of abnormal surrounding lung parenchyma, and small tumor size ($\leq$1 cm) are independently associated with likelihood of failure.

CRediT Authorship Contribution Statement

Ahmed Elsakka: Conceptualization, Methodology, Formal analysis, Investigation, Data curation, Writing-original draft preparation, Writing - review & editing, Visualization.

Elena N. Petre: Conceptualization, Methodology, Project administration, Resources, Software, Investigation, Data curation, Writing- review & editing, Visualization.

Stephen B. Solomon, Etay Ziv: Conceptualization, Methodology, Investigation, Supervision, Visualization, Writing - review & editing, Project administration.

Fourat Ridouani, Mario Ghosn, Matthew J. Bott, Bryan Husta, Maria E. Arcila, Erica Alexander: Conceptualization, Data curation, Resources, Writing - review & editing.

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