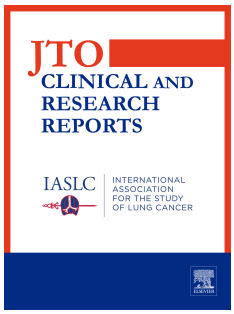

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Biopsy Method and Needle Size on Success of Next-Generation Sequencing in Non-Small Cell Lung Cancer: A Brief Report

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Disclosures-

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Abstract

Introduction:
Next generation sequencing (NGS) is essential to the care of patients with non-small cell lung cancer (NSCLC). However, NGS is dependent on adequate material from biopsy. We evaluated the impact of biopsy method and needle gauge necessary for optimizing success in tissue NGS.

Methods:
A total of 1660 formalin-fixed paraffin-embedded samples were submitted to Caris Life Sciences from 2007-2022 for tumor profiling. The results of NGS assays were linked with retrospective biopsy data for lung cancer patients treated at USC/Norris Cancer Center to create a database with the following parameters: demographics, biopsy method, tumor location (lung mass vs lymph node vs metastasis), needle gauge, number of needle passes, complications, tumor volume, DNA content, and status of NGS. Fisher's exact test and ANOVA analysis were performed to determine the impact of biopsy method and needle gauge (G).

Results:
77 CT-guided transthoracic core needle biopsies (CT-TTCN), 74 endobronchial ultrasound (EBUS)-guided transbronchial needle aspirations (TBNA), 27 bronchial forceps biopsies, and 107 surgical resections were included. 41 of 77 CT-TTCN biopsies (53.2%), 43 of 74 EBUS-TBNAs (58.1%), 22 of 27 bronchial forceps biopsies (81.5%), and 105 of 107 surgical resections (98.1%) underwent successful NGS assays. The probability of successful NGS completion for lung cancers was highest in surgical resections and bronchial forceps biopsies. Needle based biopsies were more successful when a needle larger than 20G was used. Complication rates were higher for CT-TTCN biopsies compared to EBUS-TBNA ($p<0.0001$). Overall, the DNA yield was significantly higher in EBUS-TBNA compared to CT-TTCN biopsies in primary lung sites ($p = 0.0002$). EBUS-TBNA demonstrated higher success rates in NGS compared to CT-TTCN for both primary lung lesions ($p= 0.023$) and lymph node targets ($p=0.035$).

Conclusions:
The less invasive EBUS-TBNAs demonstrated higher success rates in NGS than CT-TTCN biopsies and resulted in higher DNA concentrations. In CT-TTCN biopsies, use of 20G or smaller needles is associated with a higher risk of obtaining an inadequate specimen regardless of the number of passes taken. Surgical and bronchial forceps biopsies had highest success in achieving NGS.

Introduction:

Guidelines for lung cancer diagnosis and treatment by the National Comprehensive Cancer Network strongly recommend genomic testing for lung cancer patients at the time of diagnosis.[1] However, about only one in three lung cancer patients in the US undergo successful next generation sequencing (NGS) testing prior to initial therapy, and nearly half of patients still enter treatment with inadequate biomarker testing.[2,3]

A variety of methods are utilized for lung cancer biopsy and subsequent NGS analysis and include but are not limited to CT-guided transthoracic core needle biopsies (CT-TTCN), endobronchial ultrasound (EBUS)-guided transbronchial needle aspiration (TBNA), surgical biopsy, and bronchial forceps biopsy. The selection of biopsy method is multi-factorial and includes tumor location, tumor size, and local availability of the specialist to perform the techniques. The selection of biopsy method weighs risks and benefits including cost, convenience, and complication rate. One factor that may impact selection of method is the probability that biomarker testing will produce the quantity and quality of the tumor sample size necessary to inform treatment decisions. The aim of our study is to determine how selection of biopsy method impacts the availability for biomarker testing using NGS.

Methods

A total of 1,660 formalin-fixed paraffin-embedded samples (FFPE) samples were submitted to Caris Life Sciences between 2007 and 2022 for NGS analysis. No pathological review of specimen adequacy was performed at the local site prior to the decision to request NGS testing. Some requests for testing were sent long after initial specimen collection and run on archival tissue. Non-lung cancer cases were excluded from the study. There were 295 patients with a diagnosis of non-small cell lung cancer (NSCLC) and 5 patients with a diagnosis of small cell lung cancer (SCLC). Three patients were found to having missing medical records and were excluded from the study. Twelve patients with other biopsy techniques including bronchial brushings, bronchial alveolar lavage, thoracentesis, and multiple sample methods were excluded due to small sample size. A retrospective chart review was conducted to abstract data from electronic medical records of eligible patients at USC/Norris Cancer Center and affiliated sites. A database was constructed and included the following parameters: demographics, biopsy method, tumor location (lung mass vs lymph node vs metastasis), needle gauge, number of passes, complications, tumor volume, DNA content, and success or failure of NGS. This study was approved by the University of Southern California Institutional Review Board. Informed consent was waived given the retrospective nature of this study.

Rapid on-site evaluation (ROSE) was performed across all EBUS, transthoracic, and bronchial forceps biopsies. The tumor samples underwent comprehensive genomic profiling with Next Generation Sequencing. DNA analysis was done either through a 592 Gene Panel using Next Seq or whole exome sequencing using NovaSeq. RNA analysis was either done by utilizing an Archer FusionPlex Solid Tumor Panel through ArcherDX and whole transcriptome sequencing using NovaSeq. All FFPE samples were microdissected to allow for tumor enrichment and yield a tumor DNA composition of ≥ 20%. NGS success was defined as a complete analysis of DNA, RNA, and protein.

Statistical analysis was performed using Graphpad Prism 9. A 95% confidence interval was used to calculate each parameter comparison. Fisher's exact test and ANOVA were used to assess the likelihood of success of NGS according to biopsy method and volume of tissue sample. Biopsies collected using needles were categorized according to needle gauge. Complications were classified as minor or major. Minor complications required no intervention or just an overnight stay for observation. Major complications required intervention or longer hospital stay.

Results

Patient distribution and demographics including cancer subtype, age, gender, and race are shown in Table 1. Of 285 lung cancer patients, there were 77 CT-TTCN biopsies, 74 EBUS-TBNA, 27 bronchial forceps biopsies, and 107 surgical resections. Table 2 summarizes the next-generation sequencing successes and failure rates between different biopsy methods and biopsy location. The probability of successful NGS completion for lung cancers was statistically significant across CT-TTCN biopsy gauge sizes with 90% success rate (18/20) of NGS in 18G and 33.3% success rate (15/45) in 20G ($p<0.0001$) (Figure 1a).

DNA yield between biopsy methods

The DNA yield were subdivided between biopsy methods and biopsy location (Figure 1b). For CT-TTCN biopsies, the DNA yield was 14.8 +/- 11.5 ug/uL for primary lung lesions, 40.5 +/- 13.8 ug/uL for accessible lymph nodes, and 39.7 +/-18 ug/uL for extra thoracic non-lymph node metastatic sites. For EBUS-TBNA, the DNA yield was 43.9 +/- 19.4 ug/uL for mediastinal and hilar lymph nodes, and 41.4 +/- 16.4 ug/uL for accessible central lung lesions. For transbronchial forceps biopsies, the diagnostic yield was 44 +/- 14.2 ug/uL for primary lung tumors, compared to 46.3 +/- 13 ug/uL by surgical wedge resections. Overall, the DNA yield was significantly higher in EBUS-TBNA compared to CT-TTCN biopsies in primary lung sites ($p = 0.0002$).

Number of passes between CT-TTCN biopsies *and EBUS-TBNAs*

There was no significant difference in number of passes between needle gauge sizes in both CT-TTCN biopsies and EBUS-TBNA. For CT-TTCN biopsies, the mean number of needle passes was 4.3 +/- 2.0 for primary lung lesions, 3.3 +/- 0.8 for lymph nodes, and 3.7 +/- 1.7 for extra thoracic non-lymph node metastatic sites. For EBUS-TBNA, the mean number of needle passes was 5.6 +/- 2.7 for lymph nodes, and 5.1 +/- 3.6 for central lesions. Moreover, the number of passes was significantly higher in EBUS-TBNA compared to CT-TTCN biopsies in lymph nodes ($p=0.036$).

Complications

In CT-TTCN biopsies, the most common complications were pneumothorax and hemorrhage. The incidence of pneumothorax was 18.2% (14/77) while the incidence of hemorrhage was 2.6% (2/77). There was 1 major complication of minor-post biopsy hemorrhage with pneumothorax, which resolved following chest tube placement. Complication rate for CT-TTCN biopsies with a 18G needle was 10% (2/20) compared to 35.6% (16/45) with a 20G needle ($p=0.04$).

In EBUS-TBNA, there was 1 major complication of bleeding, respiratory failure, and hemoptysis, which resolved following intubation for several days. Overall complication rate for CT-TTCN biopsies and EBUS-TBNA were 26% (20/77) and 1.4% (1/74) respectively ($p<0.0001$).

Discussion

Our study concludes EBUS-TBNA demonstrated higher success rates in NGS compared to CT-TTCN. Furthermore, CT-TTCN biopsies with a larger needle yielded strikingly higher success rate (90% vs 33%) in NGS compared with using smaller needles. Overall complication rate was higher for CT-TTCN biopsies compared to EBUS-TBNA, but this was not driven by the patients in whom larger biopsy needles were used.

Others have found similar results with regard to the impact of biopsy method on biomarker success, but have not categorized success with regard to biopsy needle size.[4-7] While surgical resections and forceps biopsy are clearly the best method for obtaining tissue, not all patients have these specimens available or obtainable at diagnosis and often, needle biopsy is the preferred method based on anatomic and logistic considerations. We found that the failure rate with non-coring needles was much higher than a prior study by Zheng et al., which determined that surgical biopsy specimens had lower failure rates (0.7%) compared to small biopsy (5.8%) and fine needle aspiration (3.1%) specimens.[7] The increased failure rate from our study may be due to the increased requirements of tumor content necessary to do whole exome and whole transcriptome analysis compared to the hotspot panel utilized in the prior study.

DNA yields were similar for all biopsy types in large part because the DNA yield is dependent on the volume of tissue that undergoes microdissection and is then submitted for sequencing. Pathologists will perform this task to obtain the tissue required. In this case of our study, this was in the range of 40-50 ug/uL for all biopsy methods except for CT-TTCN biopsies of lungs, which typically produced under 20 ug/uL of material. Most methodologies recommend a total input of 50 ng DNA.[8] In contrast, the expanded panel in this study requires input of 60 ng. EBUS-TBNA yielded significantly higher DNA concentration from lung sites as compared to CT-TTCN biopsies along with fewer complications in our study, which may suggest that EBUS-TBNA biopsy method is preferable to CT-TTCN biopsy. This is in contrast to prior work by Yao et al. which found no significant difference in complication rates between EBUS-TBNA and CT-TTCN biopsies.[9]

Our paradoxical finding of lower complications rate with larger needles is explained by differences in the biopsy target in both groups. All 20 CT-TTCN biopsies in our series utilizing a 18G needle were performed on a non-lung site, such as an adrenal gland or lymph node, which may explain the lower complication rate in the 18G needle group. However, others have found the larger needles safe in lung. Elshafee et al. found that CT-guided lung biopsies with a 18G needle is a safe diagnostic technique with minor complication rate of 45.6% and major complication rate of 8%.[10] More recently, advances in newer techniques, such as cryobiopsy with radial-endobronchial ultrasound (Cryo-Radial), have been shown to demonstrate similar yield as CT-TTCN biopsies in peripheral lung lesions at a lower major complication rate of 4.2%.[11]

This study was retrospective and our data is limited to information available in the medical records. A prospective study of biopsy using different needle sizes and types would be required to definitively show the relative benefit to larger needles. As a single institution study, the number of physicians carrying out these procedures was small and the findings may not be applicable along the full range of skills and experience levels that exist in all centers caring for

lung cancer patients. Similar analysis by other centers is needed to confirm or refute our findings.

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Table 1. Patient characteristics.

	Total (n = 285)
Non-Small Cell Lung Cancer (NSCLC)	282 (98.9%)
Small Cell Lung Cancer (SCLC)	3 (1.1%)
Male	159 (55.8%)
Female	126 (44.2%)
Race	
Asian	90 (31.6%)
Black	19 (6.6%)
Native Hawaiian or Pacific Islander	2 (0.7%)
White	114 (40%)
Other	60 (21.1%)
Ethnicity	
Hispanic	29 (10.2%)
Non-Hispanic	248 (87%)
Unknown	8 (2.8%)

Table 2. Comparison between biopsy method/location and NGS success. NGS success was defined as complete molecular imaging (MI) profile. NGS failure was defined as limited tissue, partial quality not sufficient (QNS), or QNS. Complete MI Profile = all Whole Exome Sequencing (WES)/ 592 gene panel + Whole Transcriptome Sequencing (WTS)/ Archer panel + IHC were performed. Limited Tissue = either WES/592 gene panel or WTS/ archer panel or IHC or combination of 2. Partial QNS = either WES/592 gene panel or WTS/ archer panel or IHC or combination of 2. QNS= quantity or quality not sufficient for any testing to be performed.

		Next-Generation Sequencing Status			
Biopsy Type		**Complete MI Profile**	**Limited Tissue**	**Partial QNS**	**QNS**
Transthoracic (n=77)	**Non-Lung Site (n=25)**	19 (76.0%)	4 (16.0%)	1 (4.0%)	1 (4.0%)
	Lung (n=45)	15 (33.3%)	20 (44.4%)	2 (4.4%)	8 (17.8%)
	Lymph Node (n=7)	7	0	0	0
EBUS-TBNA (n=74)	**Lung (n=25)**	16 (64.0%)	8 (32.0%)	0	1 (4.0%)
	Lymph Node (n=49)	27 (55.1%)	17 (34.7%)	3 (6.1%)	2 (4.1%)
Surgical Resections (n=107)		105 (98.1%)	1 (1.0%)	1 (1.0%)	0
Bronch-Forceps (n=27)		22 (81.5%)	2 (7.4%)	0	3 (11.1%)

Figure 1a. Number of successful NGS and failed NGS cases in different CT-TTCN biopsy gauge sizes ($p<0.0001$).

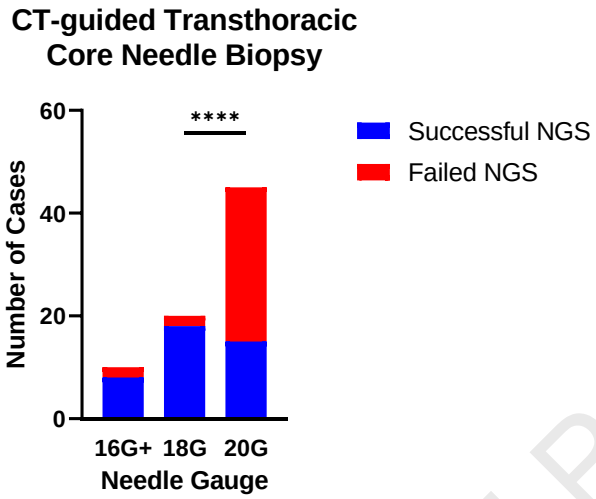
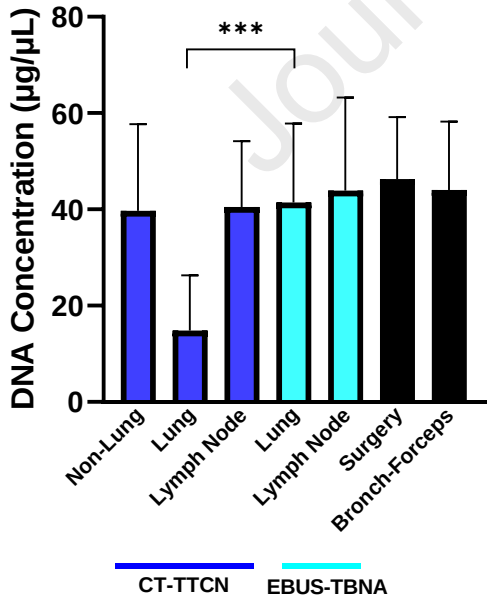


Figure 1b. DNA yield between biopsy method/location.



Supplemental Table 1. Biopsy acquisition date and NGS success.

Procedure Year	Biopsy Type	NGS Success	NGS Failure	NGS Success %
2007	Surgery	1	0	100.0%
2012	CT-TTCN	1	0	100.0%
2013	Surgery	2	0	100.0%
	CT-TTCN	1	0	100.0%
2014	Surgery	2	0	100.0%
2015	Surgery	2	0	100.0%
2016	Surgery	4	0	100.0%
	CT-TTCN	0	1	0.0%
	EBUS-TBNA	1	1	50.0%
	Bronch-Forceps	0	1	0.0%
2017	Surgery	9	0	100.0%
	CT-TTCN	7	2	77.8%
	EBUS-TBNA	4	2	66.7%
	Bronch-Forceps	1	1	50.0%
2018	Surgery	25	1	96.2%
	CT-TTCN	6	6	50.0%
	EBUS-TBNA	5	3	62.5%
	Bronch-Forceps	7	2	77.8%
2019	Surgery	12	0	100.0%
	CT-TTCN	13	9	59.1%
	EBUS-TBNA	8	7	53.3%
	Bronch-Forceps	6	0	100.0%
2020	Surgery	27	1	96.4%
	CT-TTCN	7	8	46.7%
	EBUS-TBNA	10	6	62.5%
	Bronch-Forceps	2	1	66.7%
2021	Surgery	17	0	100.0%
	CT-TTCN	5	9	35.7%
	EBUS-TBNA	15	10	60.0%
	Bronch-Forceps	6	0	100.0%
2022	Surgery	4	0	100.0%
	CT-TTCN	1	1	50.0%
	EBUS-TBNA	0	2	0.0%

CRediT Statement

Raymond Diep: Conceptualization, Methodology, Formal analysis, Investigation, Data Curation, Writing – Original Draft, Writing – Review & Editing

Madeline MacDonald: Methodology, Investigation, Data Curation, Writing – Original Draft

Ryan Cooper: Conceptualization

Anna Grzegorczyk: Conceptualization

Rastko Rakocevic: Conceptualization

Ching-Fei Chang: Methodology, Writing – Review & Editing

Angeline Uy: Writing – Review & Editing

Nicholas Cowgill: Writing – Review & Editing

Jorge Nieva: Conceptualization, Methodology, Writing – Review & Editing, Supervision