Concurrent fine needle aspirations and core needle biopsies: a comparative study of substrates for next-generation sequencing in solid organ malignancies

Sinchita Roy-Chowdhuri[1], Hui Chen[1], Rajesh R Singh[2], Savitri Krishnamurthy[1], Keyur P Patel[2], Mark J Routbort[2], Jawad Manekia[2], Bedia A Barkoh[2], Hui Yao[3], Sharjeel Sabir[4], Russell R Broaddus[1], L Jeffrey Medeiros[2], Gregg Staerkel[1], John Stewart[1] and Rajyalakshmi Luthra[2]

[1]Department of Pathology, Division of Pathology and Laboratory Medicine, The University of Texas MD Anderson Cancer Center, Houston, TX, USA; [2]Department of Hematopathology, Division of Pathology and Laboratory Medicine, The University of Texas MD Anderson Cancer Center, Houston, TX, USA; [3]Department of Bioinformatics and Computational Biology, The University of Texas MD Anderson Cancer Center, Houston, TX, USA and [4]Department of Interventional Radiology, Division of Diagnostic Imaging, The University of Texas MD Anderson Cancer Center, Houston, TX, USA

Minimally invasive procedures, such as fine needle aspiration and core needle biopsy, are commonly used for the diagnosis in solid organ malignancies. In the era of targeted therapy, it is crucial for molecular testing to be performed on these limited volume specimens. Although several recent studies have demonstrated the utility of small biopsy specimens for molecular testing, there remains debate as to whether core needle biopsy specimens are more reliable than fine needle aspiration for molecular studies. In this study, we reviewed concurrently acquired fine needle aspiration and core needle biopsy samples ($n = 24$), and compared overall cellularity, tumor fraction, and the results of next-generation sequencing. All somatic mutations detected in core needle biopsy samples were also detected in fine needle aspiration samples. The estimated tumor fraction was significantly higher in fine needle aspiration smears than core needle biopsy samples ($P = 0.003$), whereas the overall DNA yield from smears was significantly lower than that obtained from the core needle biopsy specimens ($P = 0.01$). The normalized average amplicon coverage for the genes analyzed was significantly higher in cytology smears than paired core needle biopsy samples, with lower numbers of failed amplicons and higher overall mutation allelic frequencies seen in the former. We further evaluated 100 malignant fine needle aspiration and core needle biopsy samples, acquired concurrently, for overall cellularity and tumor fraction. Overall cellularity and tumor fraction of fine needle aspiration samples was significantly higher than concurrently acquired core needle biopsy samples ($P < 0.001$). In conclusion, we show that fine needle aspiration samples frequently provide better cellularity, higher tumor fraction, and superior sequencing metrics than concurrently acquired core needle biopsy samples. Cytologic specimens, therefore, should be better integrated into routine molecular diagnostics workflow to maximize limited tissues for clinically relevant genomic testing.
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Correspondence: Dr S Roy-Chowdhuri, MD, PhD, Department of Pathology, Division of Pathology and Laboratory Medicine, The University of Texas MD Anderson Cancer Center, 1515 Holcombe Blvd. Unit 85, Houston, TX 77030, USA.
E-mail: sroy2@mdanderson.org

Ancillary molecular testing is being used increasingly to direct personalized cancer therapy for solid organ malignancies. In a large fraction of patients, these diagnoses are established by using small tissue biopsy samples obtained through minimally invasive procedures, including fine needle aspiration and core needle biopsy. Therefore, it is critical to be able

to perform molecular studies on these small samples to screen for predictive and prognostic markers and provide therapeutic targets.

Currently, both fine needle aspiration and core needle biopsy are used for obtaining diagnostic material and the choice of sampling method is largely dictated by the size and location of the lesion, the institutional practice, and the skill and preference of the operator.[1] Fine needle aspiration samples are generally obtained using 20–25 gauge needles, with or without rapid on-site evaluation for adequacy, and are often preferred for sampling small and/or deep seated lesions, tumors adjacent to at-risk structures such as blood vessels, and in situations where the needle is passed through the bronchial or bowel wall.[2,3] Core needle biopsy samples are acquired using larger 14–20 gauge needles and provide a tissue core with preservation of tissue architecture that can be used for ancillary studies.[3] Both sampling methods provide distinct advantages and limitations that are frequently complementary. Therefore, in a large number of practices, including our own institution, both methods are utilized concurrently as a diagnostic modality in amenable lesions.

Although several studies have demonstrated the utility of cytology specimens for molecular analysis, there has been some controversy regarding the adequacy of fine needle aspiration specimens[4,5] and in most institutions a core needle biopsy specimen is preferred over an fine needle aspiration sample for molecular testing.[2,6] A few studies have addressed the adequacy of cytology fine needle aspiration samples in comparison with surgical biopsy/resection samples;[7–14] however, relatively few studies have systematically evaluated concurrently acquired fine needle aspiration and core needle biopsy samples for cellularity, tumor fraction, and downstream molecular analysis.

In this study, we reviewed 24 concurrently acquired fine needle aspiration and core needle biopsy samples to compare cellular adequacy, tumor fraction, DNA yield, and the results of next-generation sequencing mutational analysis. In addition, we reviewed 100 consecutive concurrently acquired malignant fine needle aspiration and core needle biopsy samples for overall cellularity and tumor fraction.

Materials and methods

Tumor Sample Acquisition and Processing

The institutional pathology database was searched for all patients undergoing in-house concurrent image-guided percutaneous fine needle aspiration and core needle biopsy sampling, for which adequate diagnostic material was obtained for both fine needle aspiration and core needle biopsy specimens. We identified 24 consecutive malignant cases (December 2014 to January 2015) with concurrent fine needle aspiration and core needle biopsy samples that had next-generation sequencing results available on either one of the samples as part of the routine clinical work-up of the patient and with tissue available in the concurrent sample to perform matched next-generation sequencing. Two cases were rejected following initial review owing to inadequate cellularity in the fine needle aspiration sample. The selected samples included adenocarcinoma ($n = 17$), carcinoma not otherwise specified ($n = 4$), squamous cell carcinoma ($n = 2$), and melanoma ($n = 1$) from a variety of sites including lymph node ($n = 7$), liver ($n = 7$), lung ($n = 6$), soft tissue ($n = 2$), bone ($n = 1$), and adrenal gland ($n = 1$). An additional 100 consecutive malignant cases (June–July 2013) with concurrent in-house image-guided fine needle aspiration and core needle biopsy samples were also selected for morphologic evaluation. The specific diagnoses of these cases included: adenocarcinoma ($n = 51$), squamous cell carcinoma ($n = 18$), poorly differentiated carcinoma ($n = 11$), melanoma ($n = 7$), neuroendocrine carcinoma ($n = 5$), small cell carcinoma ($n = 3$), sarcoma ($n = 3$), chordoma ($n = 1$), and germ cell tumor ($n = 1$). Sites of the lesions included lung ($n = 34$), liver ($n = 23$), soft tissue ($n = 19$), lymph node ($n = 17$), bone ($n = 4$), adrenal gland ($n = 2$), and kidney ($n = 1$). The study was approved by the Institutional Review Board at our institution. Sampling was performed by interventional radiologists using ultrasound or computed tomography guidance with coaxial technique. In total, 17–19 gauge guide needles were placed adjacent to the targeted lesion and used to introduce 20–25 gauge needles for fine needle aspiration sampling (2–3 passes) and/or 16–20 gauge needles for obtaining core needle biopsy samples (3–4 cores) through the guide needle. In general, fine needle aspiration was performed first, followed by core needle biopsy sampling. However, core needle biopsy was often performed first, in subcentimeter tumors at risk of being obscured by hemorrhage from fine needle aspiration.

Direct smear preparations were made from fine needle aspirated tissue and stained using the Diff-Quik and Papanicolaou methodology (previously described[15]) for immediate assessment by a cytopathologist. After preparing smears, aspiration needles were rinsed in RPMI and cytospin or cell block preparations (ethanol/formalin-fixed and paraffin-embedded)[6] were prepared for further evaluation. Fine needle aspiration samples evaluated for this study included direct smears for all cases with cell block preparations in 53 cases. The core needle biopsy samples were fixed in 10% neutral buffered formalin, paraffin-embedded, sectioned at a thickness of 4 μm, and stained with hematoxylin and eosin (H&E).

Assessment of Sample Cellularity and Tumor Fraction

The samples were visually evaluated by light microscopy and scored as described below. As most next-generation sequencing assays require approximately between 1000 cells (eg, Ion Torrent PGM) and 5000 cells (eg, Illumina MiSeq),[16] the overall cellularity for smears scored based on the entire slide was: high, at least 1 or 2 slides with >5000 cells; moderate, 1000–5000 cells; low, <1000 cells. Overall cellularity assessments for cell blocks were not performed. Tumor fraction assessments were based upon selected tumor-rich areas within the slide. Smears and cell blocks were scored separately for tumor fraction (percentage of tumor cells relative to all cells in selected tumor-enriched areas) and when both preparations were available for review, a combined tumor fraction was generated for each case. All fine needle aspiration samples were evaluated independently by two cytopathologists (SR-C and JS). Tumor fraction assessments that were within 10% difference were averaged; any case that had >10% discordance were reviewed together by the two cytopathologists to arrive at a consensus opinion.

Core needle biopsies were scored for overall cellularity and tumor fraction based on two H&E-stained section levels (levels 1 and 8) independently by two pathologists (SR-C and HC). Cellularity for core needle biopsy samples was scored for the entire slide as: high >2000 per H&E slide; moderate 300–2000 per H&E slide; low <300 per H&E slide. The cellularity assessment criteria for core needle biopsy samples was different from the fine needle aspiration samples based on our previous experience, as (a) core needle biopsy FFPE sections are 4 µm thick and do not contain the entire nuclei resulting in lower DNA yield than the whole cells retrieved from direct smears; and (b) sectioning through the paraffin block results in loss of cellular material in subsequent sections contributing to lower DNA yield than what is estimated on a representative H&E section. Tumor fraction assessments were based upon selected tumor-rich areas within the slide. Similar to fine needle aspiration samples, any case that had discordance was reviewed again by both pathologists to reach a consensus opinion. Tumor fraction assessments within 10% difference were averaged and any case that had >10% discordance between the two pathologists were adjudicated by a re-review by both pathologists.

Mutation Testing and Data Analysis

The 24 paired fine needle aspiration and core needle biopsy samples were analyzed by next-generation sequencing using the Ion Torrent PGM (Thermo Fisher Scientific, MA) as previously described.[6,17] Fine needle aspiration smears that were selected for sequencing were photographed (representative fields) prior to extraction for digital archiving. In addition, all cases had additional smears and/or cell block material with diagnostic material retained on file for archival purposes. DNA was extracted from circled tumor-rich areas of direct smears (Diff-Quik and Papanicolaou stained) and formalin-fixed paraffin-embedded unstained tissue sections prepared from core needle biopsy paraffin blocks[17] using PicoPure (Arcturus, Mountain View, CA) and quantified using a Qubit DNA high sensitivity assay kit (Thermo Fisher Scientific) as per manufacturer's guidelines. An amplicon library was prepared using DNA from each sample using the IT Ampliseq Cancer Panel primers and quantified using the Bioanalyzer High Sensitivity DNA Chip (Agilent Technologies, Santa Clara, CA). The manufacturer-recommended input DNA for the next-generation sequencing assay is 10 ng (DNA concentration of 0.0009 µg/µl). If DNA yield was high, appropriate dilutions were made; however, if DNA yield was suboptimal, DNA was used as-is without any further dilution. Emulsion PCR was performed manually using the Ion Xpress Template Kit (Thermo Fisher Scientific) followed by manual breaking of the emulsion to isolate the ion spheres (ISPs). The quality of the DNA following PCR was measured using the Qubit IonSphere Quality control kit (Thermo Fisher Scientific). Selective ISPs with DNA were isolated and sequenced on an Ion 318 Chip. A minimum of 300 000 reads with a quality score of AQ20 (1 misaligned base per 100 bases) was used as a measure of successful sequencing with a minimum coverage of ×250 for a wild-type call.[6]

Sequence alignment and base calling was performed by Torrent Suite software v3.6.2 (Thermo Fisher Scientific) using Human Genome Build 19 (Hg19) as the reference sequence. IT Variant Caller Plugin software v3.5.59049 (Thermo Fisher Scientific) was used for the detection of variants and the variants were visualized using the Integrative Genomics Viewer (IGV). An in-house custom software (OncoSeek) was used to interface the data generated by Variant Caller with IGV to visualize the alignment and mutation detected in IGV, and annotate the sequencing results. As paired normal tissue samples were not available for analysis in all patients, variants were annotated as somatic mutations or possible germline variants based on a literature survey (including somatic mutation information as reported in COSMIC (http://cancer.sanger.ac.uk/cancergenome/projects/cosmic/) and The Single Nucleotide Polymorphism Database (dbSNP) (http://www.ncbi.nlm.nih.gov/SNP/). Any mutant allele frequency of <10% was reviewed in conjunction with the paired sample as well as in context of the tumor type and estimated tumor fraction in the sample.

Statistical Analysis

The Wilcoxon rank-sum test and McNemar's χ^2-test were used to test against the null hypothesis of no difference between the paired samples for continuous variable and categorical variable, respectively. The test results were considered to be statistically significant at a P-value < 0.05.

Results

We reviewed 24 concurrently acquired fine needle aspiration and core needle biopsy samples that had next-generation sequencing mutational analysis performed. We compared DNA yield, tumor fraction, and the results of next-generation sequencing in these samples, including a variety of sequencing metrics, such as variant calls, mutation allelic frequencies, average amplicon coverage, and number of failed amplicons.

The estimated tumor fraction was significantly higher in fine needle aspiration smears than core needle biopsy samples ($P = 0.003$); however, the overall DNA yield from smears was significantly lower than that obtained from core needle biopsy specimens ($P = 0.01$) (Table 1). All somatic mutations detected in core needle biopsy samples were detected in concurrent fine needle aspiration samples (Table 2). Details of specific mutations can be found in Supplementary Table 1. The overall mutation allelic frequencies observed in fine needle aspiration samples were higher than those in the concurrent core needle biopsy samples (Figure 1 and Table 2). To evaluate the quality of sequencing for the paired samples, we compared the average amplicon coverage by normalizing to the total number of reads in each case. The normalized average amplicon coverage for all 50 genes analyzed, as well as that of selected genes of relevance (*BRAF, KRAS, NRAS, HRAS, EGFR, PIK3CA, KIT, PTEN*, and *TP53*) was significantly higher in fine needle aspiration samples than in paired core needle biopsy samples ($P = 0.025$ and 0.014,

respectively) (Table 3). In addition, the cytology samples had lower numbers of under-performing amplicons than the core needle biopsy samples, with an average of 0.46 failed amplicons in fine needle aspiration samples *vs* 2.25 in core needle biopsy samples (Table 3).

To further evaluate our findings, we selected an additional 100 paired samples of consecutive image-guided procedures from solid organ malignancies for morphologic review and visual estimation of overall cellularity and tumor fraction. The overall cellularity of the 100 cases reviewed was as follows: fine needle aspiration smears, high: 60%; moderate: 33%; low: 7%, *vs* core needle biopsy samples high: 15%; moderate: 58%; low: 27% (Figure 2). The overall cellularity of fine needle aspiration samples was significantly higher than that of the concurrent core needle biopsy samples ($P < 0.001$) with cases of adenocarcinoma, squamous cell carcinoma, poorly differentiated carcinoma, and neuroendocrine carcinoma/small cell carcinoma typically showing higher cellularity on the cytology smears than the concurrent core needle biopsy samples (49 *vs* 4%; 78 *vs* 33%; 82 *vs* 0%; and 100 *vs* 25%, respectively) (Table 4). Metastatic tumors to the liver or lymph nodes typically showed higher cellularity on fine needle aspiration than core needle biopsy samples (Table 5).

We also compared the tumor cellularity between the fine needle aspiration and core needle biopsy samples by estimating tumor fraction based on tumor-enriched (circled) areas on the slides. Fine needle aspiration samples showed higher tumor fraction than concurrent core needle biopsy samples ($P < 0.001$), with 83% of fine needle aspiration samples having 40% or higher tumor fraction *vs* 54% of core needle biopsy samples (Figure 3 and Table 6). A significantly higher number of core needle biopsy samples had a tumor fraction < 20%, which falls below our threshold for next-generation sequencing molecular testing ($P < 0.001$) (Table 6). Half of these low tumor fraction core needle biopsy samples ($n = 7$) had 10% or less-tumor cellularity. In the subset of 14 core needle biopsy samples with a < 20% tumor fraction, the concurrent fine needle aspiration had five samples with high cellularity and a median tumor fraction of 60% (range 40–80%), seven samples with moderate cellularity and median tumor fraction of 60% (range 25–90%), and two samples with low cellularity and median tumor fraction of 38% (range 35–40%). There were also 14 core needle biopsy samples with a borderline adequate tumor fraction ranging between 20 and 25%. Of these, eight had concurrent fine needle aspiration samples with high cellularity and a median tumor fraction of 75% (range 55–90%) and six showed moderate cellularity and median tumor fraction of 43% (range 30–85%).

Table 1 Tumor fraction and DNA yield from concurrently acquired fine needle aspiration and core needle biopsy samples

	FNA smears (n = 24)	CNB (n = 24)	P-value
Tumor fraction			
Mean	54%	39%	
Median	60%	30%	*P* = 0.003
Range	25–90%	20–70%	
DNA yield			
Mean	6.6 ng/µl	17.5 ng/µl	
Median	3.6 ng/µl	12.9 ng/µl	*P* = 0.01
Range	0.36–21 ng/µl	0.27–55 ng/µl	

Abbreviations: CNB, core needle biopsy; FNA, fine needle aspiration.

Table 2 Mutational analysis characteristics of paired fine needle aspiration and core needle biopsy samples

Pt	Site	Dx	Sample	Tumor %	No. of slides used	DNA yield (ng/µl)	Input DNA (ng)	MAF #1	MAF #2	MAF #3	MAF #4	MAF #5
1	LN	CRC	FNA	80%	2 smears	14.1	13.6	APC, 65%				
			CNB	30%	12 uss	13.5	13.6	APC, 35%				
2	LN	SqCC	FNA	90%	1 smear	2.9	13.6	PIK3CA,32%				
			CNB[a]	60%	12 uss	0.6	7.7	PIK3CA,27%				
3	LN	Lung ADC	FNA	60%	4 smears	2.8	13.6	EGFR,49%	PDGFRA,39%			
			CNB	30%	10 uss	13.5	13.6	EGFR,21%	PDGFRA,45%			
4	Lung	ADC	FNA	50%	4 smears	2.7	13.6	KRAS,48%				
			CNB	20%	8 uss	4	13.6	KRAS,12%				
5	Liver	Br ADC	FNA	70%	2 smears	6.3	13.6	PIK3CA,5%	TP53,9%	CDKN2A,16%		
			CNB	60%	10 uss	1.3	13.6	PIK3CA,3%	TP53,4%	CDKN2A, 1%		
6	Liver	MM	FNA[a]	60%	3 smears	0.6	6.6	GNA11,22%				
			CNB	70%	6 uss	1.2	13.6	GNA11,24%				
7	LN	CRC	FNA	70%	1 smear	12.8	13.6	TP53,79%				
			CNB	30%	12 uss	7.2	13.6	TP53,19%				
8	Lung	ADC	FNA	60%	5 smears	1.7	13.6	KRAS,24%	PIK3CA,20%	CDKN2A,16%	TP53,7%	TP53,6%
			CNB	50%	7 uss	25.7	27.3	KRAS,20%	PIK3CA,18%	CDKN2A,31%	TP53,21%	TP53,1%
9	Liver	CRC	FNA	70%	3 smears	14.3	13.6	KRAS,38%	APC,37%	TP53,55%		
			CNB	60%	15 uss	42.6	40.9	KRAS,25%	APC,25%	TP53,33%		
10	Lung	ADC	FNA	70%	4 smears	5.7	13.6	TP53,82%				
			CNB	30%	10 uss	3.9	13.6	TP53,54%				
11	Lung	ADC	FNA	50%	2 smears	7	13.6	EGFR,24%	EGFR,27%			
			CNB	50%	10 uss	2.3	13.6	EGFR,12%	EGFR,15%			
12	Liver	CRC	FNA	40%	3 smears	4.4	13.6	PIK3CA,72%	KRAS,32%	APC,53%		
			CNB[a]	30%	12 uss	0.3	3.2	PIK3CA,48%	KRAS,22%	APC,25%		
13	Adr	Lung ADC	FNA	50%	3 smears	15.6	27.3	KRAS,71%	TP53,70%			
			CNB	50%	10 uss	25.6	27.3	KRAS,31%	TP53,24%			
14	Liver	Br ADC	FNA	70%	3 smears	21	27.3	PIK3CA,49%				
			CNB	30%	10 uss	24.6	27.3	PIK3CA,60%				
15	LN	Lung ADC	FNA	30%	5 smears	18.2	27.3	ERBB2,14%	TP53,8%			
			CNB	40%	15 uss	55	54.5	ERBB2,24%	TP53,19%			
16	Bone	RCC	FNA	30%	3 smears	1.2	13.6	VHL,19%				
			CNB	30%	8 uss	10	13.6	VHL,15%				
17	ST	UroCA	FNA[a]	30%	2 smears	0.5	6.3	TP53,73%				
			CNB	22%	15 uss	9.6	13.6	TP53,25%				
18	Lung	PDC	FNA	30%	4 smears	2.3	13.6	TP53,64%	FLT3,67%			
			CNB	40%	10 uss	32.1	40.9	TP5314%	FLT3,15%			
19	Lung	ADC	FNA[a]	30%	2 smears	0.4	4.3	EGFR,27%				
			CNB	25%	8 uss	14.6	27. 3	EGFR,14%				
20	LN	Lung ADC	FNA	60%	3 smears	2.2	13.6	NRAS,63%	SMO,62%	TP53,79%		
			CNB	40%	13 uss	42.4	40.9	NRAS,53%	SMO,56%	TP53, 67%		
21	ST	UtCA	FNA	25%	3 smears	3.2	13.6	CTNNB1,7%	FBXW7,7%	TP53,1%		
			CNB	30%	8 uss	24.8	27.3	CTNNB1,17%	FBXW7,17%	TP53,9%		
22	Liver	Pr ADC	FNA	60%	5 smears	12.2	13.6	TP53,60%				
			CNB	22%	13 uss	1.5	13.6	TP53,34%				
23	LN	Br ADC	FNA	60%	3 smears	4	13.6	PIK3CA,48%				
			CNB	25%	10 uss	51	54.5	PIK3CA,16%				
24	Liver	SqCC	FNA	50%	3 smears	1.6	13.6	TP53,33%				
			CNB	50%	5 uss	12.3	13.6	TP53,69%				

Abbreviations: ADC adenocarcinoma; Adr, adrenal gland; Br CA, breast carcinoma; CNB, core needle biopsy; CRC, colorectal adenocarcinoma; Dx, diagnosis; FNA, fine needle aspiration; LN, lymph node; MAF, mutation allelic frequency; MM, malignant melanoma; PDC, poorly differentiated carcinoma; Pr ADC prostatic adenocarcinoma; Pt, Patient; RCC, renal cell carcinoma; SqCC, squamous cell carcinoma; ST, soft tissue; UroCA, urothelial carcinoma; uss, unstained slides; UtCA, uterine endometrial carcinoma.
Additional details of specific mutations detected in Supplementary Table 1.
[a]Samples with < 10 ng input DNA.

Discussion

With advances in radiologic imaging techniques, the ability to detect and target smaller lesions using minimally invasive procedures has improved substantially. Although the volume of tissue acquired through these procedures is typically limited, the repertoire of molecular tests for predictive and prognostic markers necessary for clinical management is constantly increasing.[18–23] Therefore, it is critical to judiciously utilize these limited tissue specimens for ancillary testing, including next-generation sequencing, to provide clinically relevant information.

Minimally invasive sampling methods including fine needle aspiration and core needle biopsy are frequently complementary and used concurrently as a diagnostic modality in a large number of institutions. Several studies have evaluated the feasibility of using cytologic fine needle aspiration samples for molecular testing and recent reviews have highlighted the advantages of cytologic specimens, especially non-formalin-fixed preparations, for molecular analysis.[2,4,18,20] Aspiration cytology specimens can provide rapid on-site evaluation for adequacy assessments at the time of the procedure enabling acquisition of a better sample with appropriate triaging.[20,24–29] Most cytologic specimen preparations, with the exception of cell blocks, are not formalin-fixed and therefore provide better quality nucleic acids without cross-linking artifact.[30,31] In addition, aspirates of bone metastases are not subject to harsh decalcifying agents and provide a better source for nucleic-cid retrieval than decalcified histologic tissue.[32] Despite these distinct advantages, historically cytologic specimens have been underutilized for molecular studies. Reasons that may explain the underutilization of fine needle aspiration specimens include: (1) reluctance of many laboratories to validate a wide variety of cytologic specimen preparations (eg, direct smears, cytospin preparations, liquid-based cytology) for molecular assays;[6,16,18] (2) reluctance of cytopathologists to sacrifice irretrievable archival cytologic material (eg, direct smears);[6,16] and (3) a general misconception among the clinical and pathology community regarding the potential for utilization of cytologic material for molecular testing.[4,18,33]

In this study, we reviewed 24 paired fine needle aspiration and core needle biopsy samples to compare next-generation sequencing in these limited volume samples. In addition, we morphologically evaluated 100 consecutive concurrently acquired fine needle aspiration and core needle biopsy malignant samples from image-guided procedures to compare overall cellularity and tumor fraction. Our results demonstrate that the overall cellularity of fine needle aspiration samples was significantly higher than the concurrent core needle biopsy samples, suggesting that aspiration cytology frequently provides higher cellularity samples than
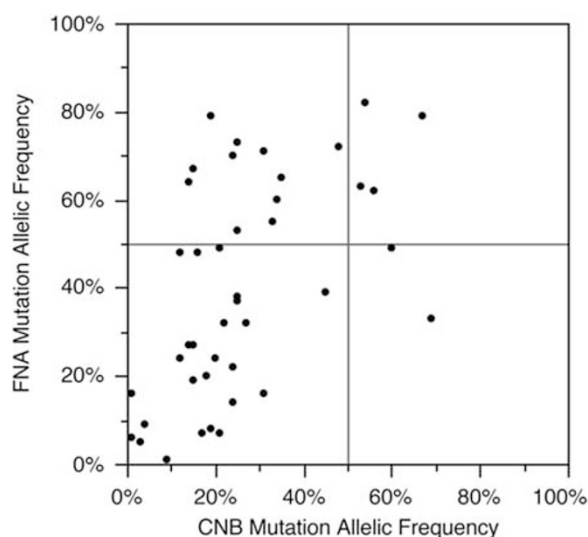

Figure 1 Comparison of concurrently acquired fine needle aspiration (FNA) and core needle biopsy (CNB) samples ($n = 24$), analyzed by next-generation sequencing (NGS).

needle biopsies for ancillary studies. Tumors with dyscohesive cells (eg, poorly differentiated carcinomas) and with extensive necrosis frequently yield high cellularity fine needle aspiration samples. Furthermore, our findings show that the tumor fraction in fine needle aspiration samples was significantly higher than that of the concurrent core needle biopsy samples. This finding reinforces the fact that specimens acquired via aspiration are highly enriched in tumor cells owing to the lower proportion of interfering stromal cells that are obtained in core needle biopsy samples. This is particularly true in tumors with increased amounts of sclerosis, eg, post-therapy cases with chemo/radiation changes.

A higher fraction of core needle biopsy samples in this study, ~ 14%, failed to meet the minimum tumor fraction threshold of 20% for the next-generation sequencing assay. The concurrent fine needle aspiration sample in all these cases was deemed adequate (by cellularity and tumor fraction) for molecular testing. Review of the cases with subcentimeter nodules ($n = 7$) where the interventional radiologist may have performed the core needle biopsy prior to the fine needle aspiration, did not show any difference in cellularity or tumor fraction when compared to the cases with larger sized tumors (data not shown), indicating that the order of sampling may not necessarily affect the cellularity and/or tumor fraction of samples. Furthermore, the results of this study showed that cytologic smears from fine needle aspiration samples provided better sequencing metrics, despite lower DNA yields following tissue extraction. At our institution, the cytopathologist attempts to enrich for tumor content by delineating tumor-rich areas on the smears for manual microdissection. This approach is more

Table 3 Next-generation sequencing metrics of paired fine needle aspiration and core needle biopsy samples

| | Cytology FNA | | | | Surgical CNB | | | |
| | | | Average coverage | | | | Average coverage | |
Patient	Failed amplicons	Total reads	All genes[a]	Selected genes[b]	Failed amplicons	Total reads	All genes[a]	Selected genes[b]
1	0	488 774	1900	1896	1	449 703	1812	1866
2	0	430 929	1812	2073	1	458 819	1436	1681
3	0	830 160	3453	4462	0	539 850	2365	2612
4	0	515 876	2277	2368	0	648 144	2265	2361
5	0	377 856	1439	1523	0	557 950	2266	2506
6	0	531 099	2326	2393	1	340 523	1145	1196
7	0	454 462	1884	2076	1	613 193	2568	2746
8	2	436 298	1612	1851	2	504 483	1372	1466
9	0	520 394	2252	2582	0	331 753	2602	2851
10	1	528 901	2358	2178	1	482 935	2123	2124
11	0	551 501	2330	2634	2	483 501	1524	1712
12	0	446 648	1940	2014	6	205 109	860	919
13	1	604 682	2688	2541	2	289 770	1221	1248
14	0	617 246	2786	2771	2	381 829	1664	1675
15	0	639 483	2742	2967	1	329 539	1409	1430
16	0	470 941	2097	2296	0	473 185	2054	2328
17	0	592 983	2586	3443	0	427 086	1851	2073
18	2	450 022	2022	2530	0	556 847	2444	2694
19	2	462 336	2042	2172	1	419 923	1833	1895
20	3	420 885	1878	2313	13	382 637	1500	1782
21	0	442 214	1797	1937	18	350 307	950	1102
22	0	423 767	1883	2281	0	413 526	1775	1983
23	0	388 513	1741	2020	1	448 429	1894	2066
24	0	586 757	2404	2646	1	447 056	1896	2083
Mean	0.46	509 014	2177	2415	2.25	439 004	1785	1933
s.d.	0.88	100 928	451	596	4.34	103 827	494	541

[a]Normalized average amplicon coverage for all 50 genes analyzed was higher in FNA samples $P = 0.025$. [b]Normalized average amplicon coverage for selected genes of relevance (*BRAF, KRAS, NRAS, HRAS, EGFR, PIK3CA, KIT, PTEN*, and *TP53*) was higher in FNA samples $P = 0.014$.
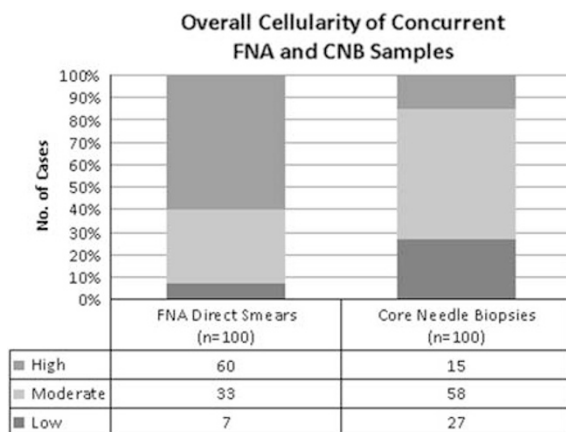
Figure 2 Comparison of overall cellularity (designated as high, moderate, and low) of fine needle aspiration (FNA) smears and concurrently acquired core needle biopsy (CNB) samples.

amenable to the three-dimensional clusters of cells on direct smears allowing for easier tumor mapping than the small cell block and core needle biopsy sections, which are usually microdissected in their entirety. This results in an increase of the tumor fraction, but at the cost of lowering the effective cellularity for tissue extraction.[2] This may explain

the significantly lower DNA yield ($P = 0.01$) and higher tumor fraction ($P = 0.003$) seen with the fine needle aspiration smears that were extracted and analyzed by next-generation sequencing. However, even with lower DNA yields, all fine needle aspiration samples had adequate DNA for successful next-generation sequencing analysis and were able to detect all mutations that were identified in the concurrent core needle biopsy sample.

Sample adequacy assessments for cellularity and tumor fraction are morphologic estimations performed by visual inspection under light microscopy and subject to inter-observer variability.[34] Mutation allelic frequency by next-generation sequencing is an independent metric that can be used to quantify tumor fraction. Our results show that most fine needle aspiration samples had higher mutation allelic frequencies that corresponded with higher estimated tumor fractions seen in the samples. Fine needle aspiration samples also had lower numbers of failed amplicons and higher average amplicon coverage when compared with the concurrent core needle biopsy samples. These results indicate that cytology smears may provide better substrates for next-generation sequencing analysis than the concurrently acquired core needle biopsy specimens. Therefore, our results argue against reflexing all molecular test requests of image-guided procedures

Table 4 Cellularity comparison of fine needle aspiration smears and core needle biopsies (*n* = 100) based on tumor type

	FNA smears (%) high cellularity	CNB (%) high cellularity
Adenocarcinoma (*n* = 51)	25 (49%)	2 (4%)
Squamous cell carcinoma (*n* = 18)	14 (78%)	6 (33%)
Poorly differentiated carcinoma (*n* = 11)	9 (82%)	0 (0%)
Melanoma (*n* = 7)	2 (29%)	3 (43%)
Neuroendocrine/small cell carcinoma (*n* = 8)	8 (100%)	2 (25%)
Others[a] (*n* = 5)	2 (40%)	2 (40%)

[a]Others: sarcoma, germ cell tumor, and chordoma.

Table 5 Cellularity comparison of fine needle aspiration smears and core needle biopsies (*n* = 100) based on site of lesion

Site of Lesion	Cellularity	FNA smears (%)	CNB (%)
Lung (*n* = 34)	High cellularity	19 (56%)	7 (21%)
	Low cellularity	3 (9%)	7 (21%)
Liver (*n* = 23)	High cellularity	17 (74%)	4 (17%)
	Low cellularity	1 (4%)	5 (22%)
Bone/soft tissue (*n* = 23)	High cellularity	8 (35%)	1 (4%)
	Low cellularity	3 (13%)	8 (35%)
Lymph node (*n* = 17)	High cellularity	13 (77%)	2 (12%)
	Low cellularity	0 (0%)	7 (41%)
Others[a] (*n* = 3)	High cellularity	3 (100%)	1 (33%)
	Low cellularity	0 (0%)	0 (0%)

[a]Others: adrenal gland and kidney.

Table 6 Morphologic estimation of tumor fraction in fine needle aspiration and concurrently acquired core needle biopsy samples

Tumor fraction	FNA (n = 100)	CNB (n = 100)
< 20%	2	14
20–39%	15	32
40–59%	19	30
60–79%	27	21
≥ 80%	37	3

Abbreviations: CNB, core needle biopsy; FNA, fine needle aspiration.
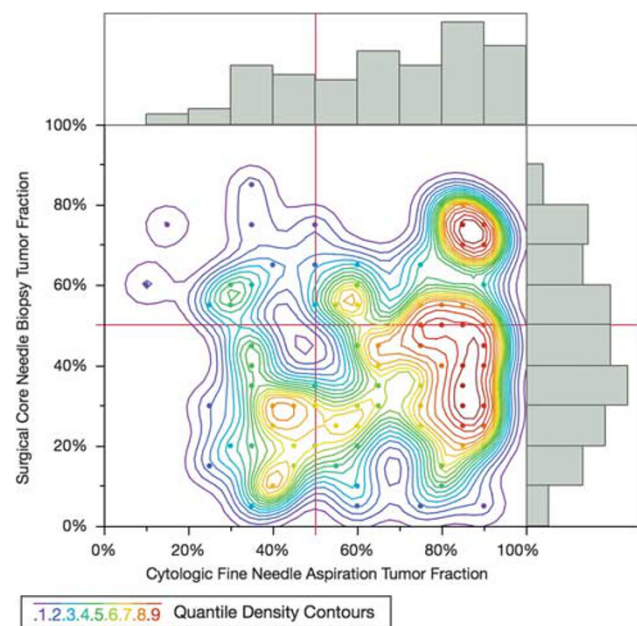
Figure 3 Density contour plot comparing estimated tumor fraction from concurrently acquired core needle biopsy and fine needle aspiration samples (*n* = 100).

to histologic biopsy specimens, especially in the context of a concurrently acquired aspiration cytology sample. Instead, molecular test requests on image-guided core needle biopsy samples need to be reviewed in conjunction with concurrently acquired fine needle aspiration specimens, if available, to select the best material for the downstream assay. The findings in this study are of considerable importance in the context of the 2013 College of American Pathologists/International Association for the Study of Lung Cancer/Association for Molecular Pathology (CAP/IASLC/AMP) clinical practice guidelines for molecular testing of lung cancer, that recommends the use of cell block sections over smears for all molecular testing. However, the guidelines are currently under revision and updated guidelines are expected to address the use of alternative cytologic preparations.

Molecular testing is now standard of care for the management of patients with several solid organ malignancies. Frequently, patients present with advanced stage disease that precludes excisional biopsy, and small specimens, including cytologic preparations, are often the only diagnostic material available for prognostic and predictive testing. Therefore, judicious use of all these limited volume specimens is needed to maximize utilization for clinically relevant biomarkers and minimize the need for re-biopsy. Many clinical trials requiring tissue for molecular studies prior to enrollment often do not accept cytology specimens as a tissue source, thus excluding patients from enrollment.[33] The reasons for excluding cytologic tissue for molecular studies are manifold, some of which have been discussed above. Standardizing specimen handling and processing techniques across cytology laboratories is critical for easier validation of cytologic specimen preparations. Digital archiving by scanning or photographing archival slides can circumvent some of the issues associated with sacrificing

cytologic smear slides for molecular testing. A better understanding and familiarity of the advantages and limitations of cytology specimens and the preanalytic factors that influence molecular testing is critical to influence this practice.

In summary, in this study we compared cytologic fine needle aspiration samples with concurrently acquired core needle biopsy samples. The results indicate that the cytology smears, as compared with core needle biopsy samples, have better cellularity, a higher tumor fraction, and better metrics by next-generation sequencing. The latter include higher mutation allelic frequency, higher average amplicon coverage, and lower numbers of under-performing amplicons. These results support the integration of cytologic specimens into routine molecular diagnostics workflow for maximizing limited tissue for clinically relevant genomic testing. The results presented also support incorporating cytologic specimens into clinical trials with the potential for increased patient enrollment and greater availability of tissues for subsequent molecular testing. In the era of precision medicine, cytopathologists can have a pivotal role in overseeing tissue sampling, processing, and appropriate triaging of small specimens to facilitate optimal utilization for molecular testing.

Disclosure/conflict of interest

The authors declare no conflict of interest.

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