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ORIGINAL ARTICLE

Comparison of the collection approaches of 2 large thyroid fine-needle aspiration practices reveals differing advantages for cytology and molecular testing adequacy rates

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Introduction At our institution, almost all thyroid fine-needle aspiration (FNA) procedures are performed by either Endocrinology or Radiology personnel. In this study, we compared the cytology and molecular adequacy rates of these 2 thyroid FNA practices, which differ on several aspects of specimen procurement.
Materials and methods All thyroid FNA specimens from Endocrinology and Radiology practices between September 2008 and December 2016 were included. Over this time frame, the molecular testing modality transitioned from polymerase chain reaction (PCR)-based (7-gene panel era) to next generation sequencing (NGS)-based (ThyroSeq era). In measuring cytology adequacy, the Bethesda System unsatisfactory rate was determined. Molecular adequacy was categorized as Optimal, Limited Thyroid Epithelial Cells, Limited Nucleic Acids, or Failed. These parameters were compared for the 2 practices.

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Results The study cohorts comprised 5810 specimens from Endocrinology and 4597 from Radiology. More Endocrinology specimens were satisfactory for cytology diagnosis than those from Radiology (94.7% versus 90.0%, $P < 0.001$). For molecular adequacy, fewer Endocrinology specimens were optimal than specimens from Radiology for both the 7-gene panel era (76.2% versus 82.9%, $P < 0.001$) and the ThyroSeq era (88.1% versus 91.9%, $P = 0.049$).

Conclusions The 2 thyroid FNA practices varied inversely in their adequacy rates for cytology and molecular testing. Had one practice been superior for both cytology and molecular adequacy, a recommendation for the method of choice would have been straightforward. However, our results show that optimization of FNA practice for the current practice of thyroid cytology requires further investigation due to the complex nature of specimen procurement.

Introduction

In conjunction with clinical evaluation and ultrasonography, fine-needle aspiration (FNA) is a common approach to evaluate thyroid nodules. In the first few decades of thyroid FNA practice, the aspirated material was used primarily for cytologic evaluation. In the past 10 years, however, molecular testing has been applied increasingly to cases of indeterminate thyroid cytology to improve risk stratification and has been advocated by the current American Thyroid Association guidelines and the Bethesda System for Reporting Thyroid Cytopathology (BSRTC).[1-3] Considering that FNA material often is used now for both cytologic and molecular testing, maximizing the yield for both tests is important.

Most of our institutional thyroid FNAs are performed in 2 settings, Endocrinology or Radiology, and these practices differ in the length and type of needles used, frequency of onsite evaluation, and application of either suction or capillary technique during the FNA procedure. To gain insight into the effects of the specimen procurement techniques, we retrospectively compared the cytology and molecular adequacy rates of the 2 practices.

Materials and methods

The study was approved as a Quality Improvement study by the University of Pittsburgh Medical Center. We enrolled all thyroid cytology cases from September 2008 to December 2016 that were collected in the Endocrinology thyroid clinic or the Radiology department. The start date corresponded to the implementation of the BSRTC. Thyroid FNA was performed using ultrasound guidance. In the Endocrinology thyroid clinic, suction was applied by the endocrinologist, who used a 1.5-in. 27-gauge and/or 25-gauge needle without a stylet. In general, a 27-gauge needle was used initially and if material was scant as assessed by onsite evaluation, then a 25-gauge needle was chosen. In the Radiology department, the radiologist or radiology physician assistant used a 3-in. needle with a stylet and the capillary technique. Onsite cytologic evaluations were performed for all cases in Endocrinology by cytotechnologists and upon request in Radiology (approximately 10% of cases) by cytopathologists, generally for cases that were previously nondiagnostic. For cases in Radiology without onsite evaluation, the standard procedure was to perform 4 passes.

The aspirated material was used for direct smears (alcohol-fixed Papanicolaou stained and air-dried Diff-Quik stained slides), monolayered ThinPrep slide processing, and molecular analysis. Specifically, direct smears were prepared for all passes from Endocrinology, and for the first 2 passes from Radiology. For both Endocrinology and Radiology, a portion of the residual material, generally corresponding to the needle rinses of the first 2 passes in both locations, was collected for molecular testing and placed in 400 μL of nucleic acid preservative solution (Roche Molecular Biochemicals, Mannheim, Germany) and stored frozen at −20°C until needed. For subsequent passes in Endocrinology, direct smears were prepared and the needle rinses were collected for ThinPrep. For subsequent passes in Radiology, the material was entirely allocated for ThinPrep.

Cytology cases were diagnosed according to the criteria and reporting format of the first edition of the BSRTC, since the study period ended before publication of the second edition.[2] The same cytotechnologists and cytopathologists prepared and signed out cases from both locations during the study period.

Molecular testing was performed reflexively on specimens with an indeterminate cytology result (ie, Atypia of Undetermined Significance/Follicular Lesion of Undetermined Significance, Follicular Neoplasm/Suspicious for a Follicular Neoplasm, or Suspicious for Malignancy) unless declined by the patient. It was also performed upon clinician request for selected cases with Unsatisfactory, Benign, and Malignant diagnoses, primarily during the 7-gene panel era. For all cases in which molecular testing was ordered, nucleic acid was isolated, as per the manufacturer's instructions, using the MagNA Pure Compact instrument (Roche) and Nucleic Acid Isolation Kit I (Roche). Mutational analyses for cases from September 2008 to September 2013 were performed using real-time polymerase chain reaction and post-polymerase chain reaction melting curve analysis (7-gene panel), and specimens that tested positive for mutations underwent direct nucleotide Sanger sequencing for confirmation.[4] For cases from October 2013 to December 2016, a next-generation sequencing panel

(ThyroSeq) was used.[5] The ratio of expression of genes specific for thyroid follicular cells (eg, *KRT7*) compared with that of a housekeeping gene was used to assess for adequacy of thyroid follicular cells, as previously described.[4]

For each specimen, cytology adequacy statements and cytology diagnoses were collected. If molecular testing was done, then molecular adequacy statements were also collected. Aspirates of cervical lymph nodes and post-thyroidectomy beds were excluded from the analysis. The molecular adequacy statements for the specimens with molecular testing were categorized based on their reported adequacy of overall nucleic acids and thyroid epithelial cells into one of the following groups: Optimal (sufficient quantity and quality of nucleic acids and sufficient expression of markers of thyroid epithelial cell differentiation), Limited Thyroid Epithelial Cells (sufficient quantity and quality of nucleic acids but limited expression of markers of thyroid epithelial cell differentiation), Limited Nucleic Acids (limited quantity or quality of nucleic acids overall or sufficient nucleic acids for some but not all of the testing), or Failed (insufficient quantity or quality of nucleic acids for any of the testing). Specimens with Limited Thyroid Epithelial Cells were also categorized by whether the cytology diagnosis mentioned lymphocytic thyroiditis to determine how frequently it was associated with this molecular adequacy category. Logistic regression was performed for assessment.

Statistical analysis of categorical variables was performed using SPSS Statistics (IBM Corp., Armonk, NY). Categorical variables were compared using Fischer's exact test, and continuous variables were compared with the Mann-Whitney U test. Logistic regression was performed to assess features associated with cytologic and molecular adequacy, using location, patient age, and nodule size as covariates. All tests were 2-sided. A *P*-value of 0.05 was considered statistically significant.

Results

The study included 10,407 thyroid FNA specimens: 55.8% (5810) from Endocrinology and 44.2% (4597) from Radiology. Differences in the FNA methods for Endocrinology and Radiology are summarized in Table 1. The median number of passes was 3 (interquartile range: 2, 4) in Endocrinology and 4 (interquartile range: 4, 4) in Radiology ($P < 0.001$). Rapid onsite evaluation was performed for 11.3% (520) of Radiology cases. Molecular testing was performed on 41.6% (2415) of Endocrinology specimens and 39.7% (1825) of Radiology specimens ($P = 0.06$). Cytologic diagnosis was performed by 12 pathologists during the course of the study, and their unsatisfactory rates are shown in Table 2. There was no significant difference in nodule size overall between the 2 locations, both of which had a median nodule size of 1.9 cm ($P = 0.13$). Similarly, among specimens that were unsatisfactory for cytology, there was also no significant difference in nodule size between the 2 locations, both of which had a median nodule size of 1.7 cm ($P = 0.89$). Repeat attempts due to unsatisfactory cytology diagnosis or suboptimal material for molecular testing represented 4.0% (231) of Endocrinology specimens and 2.8% (127) of Radiology specimens ($P \leq 0.001$) (Table 3).

For cytology diagnosis, 5.3% (308) of Endocrinology specimens and 10.0% (461) of Radiology specimens were unsatisfactory ($P < 0.001$) (Table 4). For the 520 Radiology specimens with onsite evaluation, 9.4% (49) were unsatisfactory. For molecular testing yield, in the 7-gene panel era, 76.2% (1393) of Endocrinology specimens showed Optimal adequacy, compared with 82.9% (1147) of Radiology specimens ($P < 0.001$). In the ThyroSeq era, 88.1% (516) of Endocrinology specimens showed Optimal adequacy, compared with 91.9% (406) of Radiology specimens ($P = 0.049$). Although there were no significant differences between Endocrinology and Radiology specimens in the subcategories of Failed or Limited Nucleic Acids, there were differences in the Limited Thyroid Epithelial Cells subcategory in both the 7-gene panel and ThyroSeq eras. Specifically, the 7-gene panel showed Limited Thyroid Epithelial Cells in 19.6% (359) of Endocrinology specimens and in 13.5% (186) of Radiology specimens ($P < 0.001$). ThyroSeq showed Limited Thyroid Epithelial Cells in 10.4% (61) of Endocrinology specimens and in 6.8% (30) of Radiology specimens ($P = 0.046$). Among specimens that showed Limited Thyroid Epithelial Cells, lymphocytic thyroiditis was reported in 1.7% (7) of those from Endocrinology and in 1.4% (3) of those from Radiology ($P = 1.0$). There were no significant differences between the 2 locations in the rates of any of the molecular alterations from specimens with optimal molecular adequacy (Table 5). Logistic regression showed that Endocrinology location (odds ratio 0.52), age (odds ratio 1.01), and nodule size (odds ratio 0.91) were significantly associated with an unsatisfactory for cytology diagnosis (Table 6). Meanwhile,

Table 1 Comparison of FNA methods in the endocrine clinic and in the radiology department.

Parameter	Endocrine clinic	Radiology
On-site evaluation	Routine	On request
Needle type	25- or 27-gauge, 1.5-in. without stylet	25-gauge, 3-in. with stylet
Fine-needle aspiration technique	Suction	Capillary

Table 2 Rates of unsatisfactory cytologic diagnosis by pathologist.

Pathologist	Unsatisfactory for cytology specimens n (%)	Total specimens
1	0 (0)	19
2	0 (0)	28
3	31 (4.9)	631
4	47 (5.6)	842
5	3 (5.9)	51
6	127 (6.5)	1968
7	18 (6.6)	273
8	112 (7.2)	1550
9	14 (7.9)	178
10	242 (8.2)	2950
11	133 (8.7)	1526
12	42 (10.8)	389

only Endocrinology location (odds ratio 1.52) was significantly associated with suboptimal molecular adequacy (Table 7).

Discussion

Numerous studies have assessed the degree to which different factors affect adequacy rates for thyroid FNA cytology. These factors include number of passes,[6,7] use of onsite evaluation,[8,9] operator experience,[8] nodule size or depth,[6,10] needle gauge,[11,12] use of a needle stylet,[13] cystic degeneration,[10,14] use of ultrasound guidance,[15,16] liquid-based cytology,[11,17] and aspiration versus capillary technique.[18,19] Other studies have reported adequacy rates for molecular assays of thyroid FNA.[5,20] We believe this is the first study to analyze both cytologic and molecular adequacy for 2 different specimen procurement methods. In this retrospective concurrent cohort study, we observed that despite using significantly fewer passes, the clinical practice that routinely used a 1.5-in. needle, no stylet, and an aspiration technique demonstrated significantly fewer unsatisfactory FNA results yet significantly less molecular adequacy yield, than the practice that routinely used a 3-in. needle and capillary technique.

The inverse nature of these differences suggest that a preference in the allocation of material for cytologic diagnosis and molecular testing may have played a role. For instance, it is possible that more cells remained within the bore of the 3-in. spinal needle used by Radiology than the 1.5-in. standard needle used by Endocrinology after preparing direct smears. Therefore, when rinsing the first 2 passes for molecular testing, a greater proportion of cells may be allocated to molecular testing from Radiology specimens than from Endocrinology specimens. This might be related to the greater length of the needle used by Radiology or to other physical properties of the needle. It should be emphasized that both practices in this study use needle rinses for molecular testing, and generally do not collect separate passes for molecular testing.

Of the material allocated for cytologic diagnosis, the Radiology protocol allocated relatively more material than the Endocrinology protocol for ThinPrep slides as opposed to direct smears. Nonetheless, the material was allocated similarly between cytology (direct smears of ThinPrep) and molecular testing at both locations, since molecular testing was collected from the needle rinses of the first 2 passes at both locations.

The multiplicity of variables related to procurement techniques that could not be controlled or measured precluded the determination of the precise cause for the differences. We postulate, however, that in general, the routine use of onsite evaluation favors diagnostic cytology adequacy (as prior literature has shown[7-9]) and more passes, capillary technique, and longer needle favor molecular adequacy. Nevertheless, there may be additional factors that are difficult to measure. We are anecdotally aware that in some situations, patients with nodules too deep to access, based on imaging, were referred from Endocrinology to Radiology, indicating that the overall difficulty level of Radiology FNAs may be higher. This happened only a few times per year, according to our Endocrinology colleagues. Although in theory this could partially explain the lower satisfactory cytology rate in Radiology specimens, it would not explain the higher optimal molecular adequacy in Radiology specimens. Also, considering that Endocrinology uses a standard needle without a stylet, it is possible that in some cases normal thyroid is sampled that is misinterpreted as lesional and therefore satisfactory for evaluation. The issue of contamination by normal tissue has not been adequately studied in the thyroid cytology literature, yet the endoscopic literature states that the use or omission of a stylet does not affect the tissue contamination rate.[21,22] Endocrinology's lower unsatisfactory for cytology rate

Table 3 Comparison of parameters between endocrine clinic and radiology department.

Specimen parameter	Endocrine clinic	Radiology	Total	*P*-value
Nodule size	1.9 (IQR 1.4, 2.8)	1.9 (IQR 1.4, 2.7)	1.9 (IQR 1.4, 2.7)	0.13
Median number of passes	3 (IQR 2, 4)	4 (IQR 4, 4)	4 (IQR 3, 4)	<0.001
Repeat attempt specimens	231 (4.0%)	127 (2.8%)	358 (3.4%)	<0.001
Specimens with molecular testing	2415 (41.6%)	1825 (39.7%)	4240 (40.7%)	0.06

Abbreviation: IQR, interquartile range.

Table 4 Adequacy for cytology and molecular testing, comparing the 2 thyroid FNA practices.

Diagnostic modality	Adequacy category	Location			*P*-value	*P*-value overall
		Endocrinology n (%)	Radiology n (%)	Total n (%)		
Cytologic diagnosis	Unsatisfactory	308 (5.3)	461 (10.0)	769 (7.4)	<0.001	<0.001
Molecular testing: 7-gene panel	Optimal: Sufficient nucleic acids and sufficient thyroid epithelial cells	1393 (76.2)	1147 (82.9)	2540 (79.1)	<0.001	<0.001
	Limited thyroid epithelial cells	359 (19.6)	186 (13.5)	545 (17.0)	<0.001	
	Limited nucleic acids	25 (1.4)	24 (1.7)	49 (1.5)	0.47	
	Failed: Insufficient nucleic acids	52 (2.8)	26 (1.9)	78 (2.4)	0.083	
	Total	1829 (100.0)	1383 (100.0)	3212 (100.0)		
Molecular testing: ThyroSeq	Optimal: Sufficient nucleic acids and sufficient thyroid epithelial cells	516 (88.1)	406 (91.9)	922 (89.7)	0.049	0.18
	Limited thyroid epithelial cells	61 (10.4)	30 (6.8)	91 (8.9)	0.046	
	Limited nucleic acids	6 (1.0)	5 (1.1)	11 (1.1)	1.0	
	Failed: Insufficient nucleic acids	3 (0.5)	1 (0.2)	4 (0.4)	0.64	
	Total	586 (100.0)	442 (100.0)	1028 (100.0)		

Table 5 Comparison of molecular alterations of specimens with optimal molecular adequacy.

Molecular alteration	Falk n (%)	Ultrasound n (%)	Overall n (%)	*P*-value
None	1512 (79.3)	1218 (78.4)	2730 (78.9)	0.56
Fusions	21 (1.1)	19 (1.2)	40 (1.2)	0.75
ALK/STRN	0 (0)	1 (0.1)	1 (0)	
ETV6/NTRK3	0 (0)	3 (0.2)	3 (0.1)	
PAX8/PPARG	11 (0.6)	7 (0.5)	18 (0.5)	
RET/PTC	10 (0.5)	8 (0.5)	18 (0.5)	
Mutations	370 (19.4)	311 (20.0)	681 (19.7)	0.67
BRAF K601E	14 (0.7)	5 (0.3)	19 (0.5)	
BRAF K601E & TERT	0 (0)	1 (0.1)	1 (0)	
BRAF V600E	117 (6.1)	89 (5.7)	206 (6)	
BRAF V600E & TERT	0 (0)	1 (0.1)	1 (0)	
BRAF, other	2 (0.1)	1 (0.1)	3 (0.1)	
EIF1AX	12 (0.6)	16 (1)	28 (0.8)	
EIF1AX & PTEN	2 (0.1)	0 (0)	2 (0.1)	
GNAS	2 (0.1)	1 (0.1)	3 (0.1)	
HRAS	40 (2.1)	40 (2.6)	80 (2.3)	
HRAS & EIF1AX	1 (0.1)	0 (0)	1 (0)	
KRAS	26 (1.4)	27 (1.7)	53 (1.5)	
KRAS & p53	1 (0.1)	0 (0)	1 (0)	
NRAS	115 (6)	92 (5.9)	207 (6)	
NRAS & EIF1AX	2 (0.1)	0 (0)	2 (0.1)	
NRAS & PTEN	0 (0)	1 (0.1)	1 (0)	
NRAS & TERT	0 (0)	1 (0.1)	1 (0)	
P53	0 (0)	2 (0.1)	2 (0.1)	
PIK3CA	2 (0.1)	0 (0)	2 (0.1)	
PTEN	8 (0.4)	11 (0.7)	19 (0.5)	
RET	1 (0.1)	1 (0.1)	2 (0.1)	
TERT	1 (0.1)	0 (0)	1 (0)	
THADA	6 (0.3)	2 (0.1)	8 (0.2)	
TSHR	18 (0.9)	20 (1.3)	38 (1.1)	
RNA expression	4 (0.2)	5 (0.3)	9 (0.3)	0.53
NIS overexpression	2 (0.1)	1 (0.1)	3 (0.1)	
PTH overexpression	2 (0.1)	4 (0.3)	6 (0.2)	
Total	1907 (100)	1553 (100)	3460 (100)	

Table 6 Logistic regression of variables associated with unsatisfactory for cytology diagnosis.

Variable	*P*-value	Odds ratio (95% confidence interval)
Location (Endocrinology)	<0.001	0.52 (0.44-0.60)
Age	<0.001	1.01 (1.01-1.02)
Size	0.01	0.91 (0.85-0.98)

was not explained by proportion of specimens that were repeat attempts, considering that Endocrinology had a higher proportion of repeat specimens (4.0% versus 2.8%) than Radiology.

Although adequacy for cytologic diagnosis was determined on site by cytotechnologists in Endocrinology and by cytopathologists in Radiology (when onsite evaluation was requested), adequacy was ultimately determined by the cytopathologist when signing out the case, regardless of the findings during onsite evaluation. Considering that the unsatisfactory for cytology rate of Radiology cases with onsite evaluation (by pathologists) was significantly higher than Endocrinology cases (which all had onsite evaluation by cytotechnologists [9.4% versus 5.3%]), it seems unlikely that the use of cytotechnologists rather than pathologists for onsite evaluation had a deleterious effect on cytologic adequacy.

The mutational data indicate that both locations showed a similar proportion of specimens positive for a molecular alteration (20.7% versus 21.6%). Also, there was no significant difference in the types of alterations (fusions, mutations, and RNA over-expression), arguing against the possibility that differences in cytologic adequacy might be explained by differences in molecular alterations. We note that the differences in molecular adequacy rates between the 2 thyroid FNA practices were only significant in the category of Limited Thyroid Epithelial Cells. Conceptually, this result could either result from suboptimal sampling and/or insufficient material allocated for molecular testing or from a true scarcity of thyroid epithelial cells in the sampled nodule relative to non-epithelial cells, as is often seen in lymphocytic thyroiditis. We found that lymphocytic thyroiditis was reported for very few specimens with Limited Thyroid Epithelial Cells and that there was no significant difference in the frequency of this diagnosis between the 2 practices among these specimens. Therefore, we consider it likely that these specimens resulted from suboptimal sampling and/or insufficient material allocated for molecular testing, with most of the non-epithelial cells representing circulating white blood cells as opposed to thyroid inflammation.

Table 7 Logistic regression of variables associated with suboptimal molecular adequacy.

Variable	*P*-value	Odds ratio (95% confidence interval)
Location (Endocrinology)	<0.001	1.52 (1.29-1.79)
Age	0.19	1.00 (1.00-1.01)
Size	0.08	1.06 (0.99-1.13)

Whereas Radiology had a higher rate of adequate molecular specimens, the difference in the adequacy rate of molecular specimens from Endocrinology and Radiology diminished from the 7-gene era (76.2% versus 82.9% or difference of 6.7 percentage points) to the ThyroSeq era (88.1% versus 91.9% or difference of 3.8 percentage points). Although these differences were statistically significant, the minor difference in molecular adequacies in the ThyroSeq era may be practically less important. Nonetheless, our clinicians manage a Suboptimal molecular result (Limited Thyroid Epithelial Cells or Limited Nucleic Acids) in the same way that they manage a Failed molecular result. Therefore, improvement in specimen procurement is desirable.

Had 1 procurement method been superior for both cytology and molecular adequacy, the recommendation for the method of choice would have been straightforward. However, our results show that different thyroid FNA specimen procurement techniques may affect the adequacy rates for cytology diagnosis and molecular testing independently (eg, routine use of onsite evaluation does not ensure superior adequacy for both cytology diagnosis and molecular testing). Further investigation is needed to optimize the FNA practice for the current state of thyroid cytology.

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Conflict of interest disclosures

The authors declare no conflicts of interest.

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