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ORIGINAL PAPER

Importance of FNA Technique for Decreasing Non-diagnostic Rates in Thyroid Nodules

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Abstract To identify potential interventions that may lower the high non-diagnostic rates associated with ultrasound guided (US) fine needle aspiration (FNA) biopsy of the thyroid nodule. A case series of 164 thyroid nodule US-guided FNA was identified retrospectively. The following variables were analyzed in regards to diagnostic and non-diagnostic sampling: patient age, gender, size of nodule, biopsy technique (capillary vs. aspiration), needle gauge (23 vs. 25), and physician experience. The FNA diagnosis, and final pathology, when applicable, was recorded for each sample using the Bethesda criteria. Data was analyzed using the Fisher's exact test or the chi square test. After multivariate logistic regression, capillary action was independently associated with lower non-diagnostic rates ($p = 0.01$), while increasing patient age was associated with higher non-diagnostic rates ($p = 0.018$). Physician experience ($p = 0.014$) was not independently associated with lower non-diagnostic rates. Nodules that were "cystic >50%" were significantly more likely to yield a non-diagnostic result ($p < 0.0001$). After taking into account confounding variables, including physician experience, our data reveals a statistically significant decrease in non-diagnostic rates with the use of capillary action vs. aspiration technique in US-guided FNA. A major focus in healthcare today is providing cost-effective and minimally invasive care to the patient. In the setting of a rising incidence of thyroid disease, we believe our study demonstrates the need for a prospective analysis of the relationship between technique and non-diagnostic rates.

Keywords Thyroid nodule · Fine needle aspiration · FNA · Aspiration technique · Capillary action · Non diagnostic rates

Introduction

The incidence of thyroid nodules and thyroid cancer is on the rise; the latter has more than doubled since 1973 [1]. Although 5% of women and 1% of men have palpable thyroid nodules, ultrasound will reveal a nodule in 19–67% of individuals [1]. This increased rate of detection has direct clinical, financial, and therapeutic implications. Although the rate of thyroid cancer in any one of these nodules is typically 5–15% [1], thyroid cancer, nevertheless, must be ruled out. Fine-needle aspiration biopsy (FNAB) has been shown to be the most accurate, quick, and safe means to do this [2–4]. Ultrasound guided FNAB is superior to palpation guided FNAB, insofar as it yields significantly more diagnostic samples [5–7], while also reducing the false-negative rate.

Despite the established efficacy of FNAB in the workup of thyroid masses, relatively high non-diagnostic rates continue to hinder the full potential of the FNAB. These rates for ultrasound guided FNAB in the literature range widely from 2 to 29% [8, 9]. Such non-diagnostic results increase costs, patient stress, and time to diagnosis. Most importantly, studies have shown that initial non-diagnostic samples can harbor a malignancy in 1–4% of cases [10].

Several studies in the *radiology* literature have examined the influence of needle gauge and/or biopsy technique (aspiration vs. capillary action) on diagnostic accuracy in

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ultrasound guided FNAB of neck masses using varying classification systems [11–13]. However, only one similar surgeon-led study in the *otolaryngology* literature has examined the effect of needle size on non-diagnostic rates [14]. Although Bhakti et al. reported non-diagnostic rates in a one surgeon experience with a combination of both capillary and aspiration technique [15], no surgeon-led study in the *otolaryngology* literature has differentiated between aspiration and capillary technique to analyze the relationship of technique on non-diagnostic rates. This study reports a one-surgeon experience detailing 5 years of thyroid FNAB using the Bethesda criteria. The study examines the relationship between non-diagnostic rates and FNAB technique.

Materials and Methods

A minimal risk IRB was approved in conjunction with a HIPAA waiver for informed consent. A total of 164 thyroid ultrasound guided FNAB were performed by the senior author at George Washington University Hospital form September 2009 to June 2014. Patients consisted of 33 males and 131 females. Patient age ranged from 23 to 89 years. FNA was performed on all nodules >10–15 mm, as well as nodules <10 mm that demonstrated suspicious features on ultrasound [16]. Suspicious features included microcalcifications, intranodular hypervascularity, irregular margins, hypoechoic solid nodule, and nodal involvement [16].

The following data was collected for each FNAB sample: age and gender of patient, anatomic location of biopsy, size, biopsy technique, needle gauge, adequacy of sample, FNA diagnosis, final pathologic diagnosis when applicable.

Each nodule underwent ultrasound imaging by the senior author. Echogenicity, vascular pattern, size, location, margins, composition, and calcification were recorded (Table 1). Nodules were characterized as either > or <50% cystic based on ultrasound findings.

Ultrasound guided FNA was performed by the senior author with either a 23 or 25-guage needle. Informed consent was obtained for all patients who underwent FNAB. The needle was used to aspirate or draw up cellular material via capillary action from the solid portion of the nodule. The aspirated material was used to form air-dried smears by forcefully expunging it onto a thin prep slide, smearing it with a second slide, and then allowing it to air dry. Material drawn up by capillary action was gently spread along the horizontal length of the thin prep slide, smeared and then allowed to air dry. All biopsies were performed without an on-site cytopathologist. Slides were sent to cytology department for Diff-Quik staining.

The following needle gauge and biopsy techniques were used during 9/2009-10/2012, 10/2012-10/2013, and 10/2013-06//2014: 23-gauge with aspiration, 25-gauge with aspiration, and 23-gauge with capillary action, respectively. Technique was altered during these time periods in an attempt to lower non-diagnostic rates. All biopsies were performed using 4 passes.

Table 1 Study demographic and ultrasound characteristics of thyroid nodules in relation to diagnostic and non-diagnostic results

Variable	Non dx (n = 44)	Dx (n = 120)	p value
Sex female	31 (70.5%)	100 (84.0%)	0.053
Age	49.9 ± 14.6	54.8 ± 15.6	0.07
Nodule size			0.14
<1 cm	4 (9.1%)	4 (3.3%)	
1–3 cm	28 (63.6%)	68 (56.7%)	
>3 cm	12 (27.3%)	48 (40.0%)	
Nodule margin			0.77
Indistinct	13 (29.6%)	43 (35.8%)	
Well defined	31 (70.5%)	77 (64.2%)	
Vascularity			0.27
Hypervascular	5 (11.4%)	17 (14.2%)	
Hypovascular	1 (2.3%)	1 (0.83%)	
Normovascular	38 (86.4%)	102 (85%)	
Calcification pattern			0.50
No	30 (68.2%)	89 (74.2%)	
Macrocalcification	2 (4.6%)	8 (6.7%)	
Microcalcification	12 (27.3%)	23 (19.2%)	
Nodule composition			**<0.0001**
Cystic <50%	9 (20.5%)	47 (39.2%)	
Cystic >50%	13 (29.6%)	5 (4.2%)	
Solid	22 (50.0%)	68 (56.7%)	
Echogenicity			0.13
Heterogeneous	6 (14.0%)	35 (29.4%)	
Hypoechogenic	33 (76.7%)	76 (63.9%)	
Isoechogenic	4 (9.3%)	8 (6.7%)	
Posterior location	2 (4.6%)	2 (1.7%)	0.23
Needle gauge			**0.01**
23	27 (61.4%)	97 (80.8%)	
25	17 (38.6%)	23 (19.2%)	
Technique			**0.01**
Aspiration	38 (86.4%)	79 (65.8%)	
Capillary	6 (13.6%)	41 (34.2%)	

Statistically significant values ($p < 0.05$) are given in bold

Cytopathology results were retrospectively reviewed and classified based on the 6-tiered Bethesda criteria [10]. Results were classified as: non-diagnostic, benign, atypical cells of undetermined significance/follicular lesion of undetermined significance, suspicious for follicular neoplasm, suspicious for malignancy, and malignant (Table 2). Specifically, "non-diagnostic" results included nodules that were "completely cystic, virtually acellular (<6 groups of at least 10 cells) or otherwise unrevealing due to blood or artifact [10]." Although the cytopathology system at our university

Table 2 Distribution of FNA diagnoses of study compared against literature values

FNAC category	Study (n)	Study (%)	Literature review [10]
Non-diagnostic	44	26.8	2–29% [8, 9]
Benign	95	57.9	59.3%
AUS/FLUS	10	6.1	9.6%
SFN	5	3.0	10.1%
SFM	1	0.6	2.7%
Malignant	9	5.5	5.4%
Total	164	100	100

AUS atypia of unspecified significance, *FLUS* follicular neoplasm of unspecified significance, *SFM* suspicious for malignancy, *SFN* suspicious for follicular neoplasm

Table 3 Non-diagnostic rates by year in practice

Year	Non-diagnostic	Diagnostic	p value
2009	0 (0%)	6 (100%)	**0.014**
2010	6 (32%)	13 (68%)	
2011	3 (14%)	18 (86%)	
2012	13 (34%)	25 (66%)	
2013	20 (39%)	32 (62%)	
2014	2 (7%)	26 (93%)	

Statistically significant values ($p < 0.05$) are given in bold

varied slightly from the Bethesda system, we were able to extrapolate our cytopathology classifications.

Patient demographics and ultrasound features of thyroid nodules were compared in non-diagnostic versus diagnostic cases using a 2-tailed independent-groups t test (continuous data), chi square test or Fisher's exact test (for categorical data). Multivariate logistic regression analysis was performed to determine independent factors that predicted non-diagnostic cases after accounting for all covariates that were potentially associated with insufficiency (based on having univariate $p < 0.10$). SAS (version 9.3, Cary, NC) was used for all data analysis with $p < 0.05$ considered statistically significant.

Results

Nodule composition ($p < 0.0001$), needle gauge ($p = 0.01$), and technique ($p = 0.01$) were significantly associated with non-diagnostic rate (Tables 1, 3). The non-diagnostic rate was lowest for nodules that were "cystic <50%" (16%) and highest for "cystic >50%" (72%). The non-diagnostic rates for 23- and 25-gauge needles were 22 and 43%, respectively. Aspiration technique resulted in a non-diagnostic rate of 32%, while the rate for capillary action was 13%. The non-diagnostic rate by year of surgeon was 0, 32, 14, 34, 39, and 7% for years 2009 through 2014, respectively (Table 3). The non-diagnostic rate according to year by technique was 27, 43, and 13% (Table 4).

After multivariate logistic regression, predictors with significant independent effects included age, nodule composition, and technique (Table 5). The prediction model was significant ($p < 0.0001$) with a strong effect size (pseudo-$R^2 = 0.25$) and good prediction accuracy ($c = 0.82$). Each year of patient age raised the odds of insufficiency by 4% (OR 1.04 [95% CI 1.01–1.07], $p = 0.018$). Nodules that were "cystic >50%" had 13 times the odds of being non-diagnostic, compared to solid nodules. Nodules biopsied via aspiration had 21 times the odds of being non-diagnostic compared to nodules that underwent capillary action, after accounting for the other covariates.

Of all nodules with FNAB results (n = 164), 52 underwent surgical sampling. The malignancy rate was 26.9%, as 38 nodules were benign and 14 were malignant. Overall sensitivity, specificity, PPV, NPV, and accuracy for

Table 5 Multivariate regression analysis—variables independently associated with non-diagnostic rates

Variable	Non dx (n = 44)	Dx (n = 120)	p value
Age	49.9 ± 14.6	54.8 ± 15.6	**0.018**
Technique			**0.03**
Aspiration	38 (86.4%)	79 (65.8%)	
Capillary	6 (13.6%)	41 (34.2%)	
Nodule composition			**0.0002**
Cystic <50%	9 (20.5%)	47 (39.2%)	
Cystic >50%	13 (29.6%)	5 (4.2%)	
Solid	22 (50.0%)	68 (56.7%)	

Statistically significant values ($p < 0.05$) are given in bold

Table 4 Non-diagnostic rates—years by technique

Year	Technique	Non-diagnostic	Diagnostic	p value
9/2009–10/2012	23-Gauge w/aspiration	21 (27.3%)	56 (72.7%)	**0.011**
10/2012–10/2013	25-Gauge w/aspiration	17 (42.5%)	23 (57.5%)	
10/2013–06/2014	23-Gauge w/capillary	6 (12.8%)	41 (87.2%)	

Statistically significant values ($p < 0.05$) are given in bold

detecting malignancy were 64, 100, 100, 88, and 90%, respectively. "Suspicious for follicular neoplasm" and "non-diagnostic" were excluded in this analysis.

Discussion

FNAB has been shown to be an efficacious primary diagnostic tool in the workup of head and neck masses. Indeed, the accuracy of detecting benign and malignant lesions in the present study was 90%. The sensitivity, specificity, PPV, and NPV for detecting benign vs. malignant lesions was 64, 100, 100, 88%, respectively. The high sensitivity, specificity, and accuracy in the present study support the use of FNAB as a primary screening tool in the clinical setting. Furthermore, these values are replicated in the literature. Wong et al. evaluated 13 similar studies in the context of the Bethesda criteria, stating that ultrasound guided FNAB was highly sensitive (84–94%) and specific (98–99%) in detecting malignancy [17]. A separate meta-analysis on the Bethesda criteria reported a PPV and NPV of 98.6 and 96.3% for the identification of benign and malignant lesions [8].

The literature, and the present study, support the efficacy of ultrasound guided FNAB, but non-diagnostic rates remain high (2–29%), despite the implementation of the Bethesda criteria in 2007 [8, 9]. The wide range may be due to the use of different biopsy techniques and varying operator experience among the studies. Lack of standardization is not an issue due to the implementation of the Bethesda criteria.

In an attempt to improve diagnostic adequacy of FNAB samples, we focused on the association between non-diagnostic rates, and needle gauge/biopsy technique. Both a 23-gauge needle ($p=0.01$) and capillary action ($p=0.01$) were associated with a statistically significant decrease in non-diagnostic rates when compared to 25-gauge needle and aspiration technique (Table 1). Again, several studies in the non-otolaryngology literature have examined the relationship between biopsy technique and non-diagnostic rates. Most of these studies, including Tauro et al. and Tubulin et al., have found no statistically significant difference in these rates based on aspiration versus capillary action [18–22]. Nevertheless, Tauro et al. concluded that aspiration may be better for largely acellular nodules, whereas capillary action may be better for cellular lesions [21]. Tubulin et al. concluded that capillary action is superior to aspiration insofar as it is easier to learn and faster to perform [19].

Degirmenci et al. is an exception to this trend, as they did note a statistically significant decrease in non-diagnostic rates with the use of a capillary action over aspiration [13]. Dey et al. also reported that capillary action could provide better sampling than aspiration [23]. Most recently, Zhou et al. suggested that choice of technique should be based on the size of the nodule [24]. Aspiration provided lower non-diagnostic rates in nodules that were 5.1 to 10 mm and >20 mm in their study, whereas aspiration and capillary action were of equal diagnostic benefit in the remainder of the nodules.

Similarly, most studies do not report an association between non-diagnostic rates and needle size [11, 12, 14, 25], although smaller needle size has been shown to decrease pain [11] and provide more cellular material [26]. Naiim et al. reported a decrease in non-diagnostic specimens when the number of passes was increased from 1 to 3, but related no difference when 2 or 3 passes were used [11].

It is important to note the studies that have examined the relationship between technique and non-diagnostic rates have done so prior to the implementation of the Bethesda criteria. In fact, to the best of our knowledge, ours is the only study in the literature to have examined the association between non-diagnostic rates and technique in the context of the Bethesda criteria.

The present study demonstrates a clear decrease in non-diagnostic rates with capillary action and thus may provide guidance for the novice clinician interested in implementing ultrasound guided FNAB. Capillary action technique has been demonstrated to provide specimens with greater cellularity and higher quality diagnostic material.

Multivariate logistic regression analysis was performed to determine independent factors predictive of non-diagnostic results after accounting for all covariates that were potentially associated with non-diagnostic results. Capillary action and age, but not needle gauge, was found to provide a significant decrease in non-diagnostic results after accounting for confounding variables. Similarly, nodules "cystic >50%," as well as samples from older patients, were more likely to yield a non-diagnostic cytopathology result.

Interestingly, multivariate logistic regression analysis demonstrated that physician experience was not an independent factor for the degree of diagnostic adequacy, although there was a statistically significant relationship between physician experience and non-diagnostic rates ($p=0.014$). There are likely two reasons for this observation. First, as explained previously, the use of aspiration with a 25-gauge needle during the majority of 2012 and 2013 resulted in a higher non-diagnostic rate compared to 2009–2012. This created a non-linear decrease in non-diagnostic rates across the years of the study, which prevented physician experience from being acknowledged as an independent factor in the degree of non-diagnostic samples. Second, at the cessation of the study, the most recent year, 2014, in the physician's career was still incomplete. It is likely, given the trend in 2014, that a greater sample size incorporating the remainder of 2014 would demonstrate physician experience to be an independent factor in the prediction of non-diagnostic results.

There are several limitations to this study. The retrospective design of the study can lead to selection bias. Moreover, only the nodules that underwent surgery were used to calculate sensitivity, specificity, and accuracy. The decision to proceed with surgery was favored in patients with a worrisome presentation, or suspicious features on ultrasound and/or FNAB. The FNAB reporting system used at our institution is not the same as the Bethesda system, although it is sufficiently similar to allow for easy extrapolation. Additionally, the results of this study may not be generalizable to the public as it represents a small, single surgeon's patient population at a tertiary care center with cases that may be different in complexity compared to what is usually seen by community practitioners; it is possible that given the complexity, cases referred were more likely to be non-diagnostic than what might be seen by in the community. Finally, due to the retrospective nature of study, several different pathologists reviewed the cytology results, which may have led to increased inter-observer variability. The potential confounding relationship between multiple different variables, including physician experience, and non-diagnostic rates was not a limitation, as it was corrected for using logistic regression analysis.

Conclusion

After taking into account confounding variables, including physician experience, our data reveals a statistically significant decrease in non-diagnostic rates with the use of capillary action compared to aspiration technique in US-guided FNAB. A major focus in health care today is providing cost-effective, minimally invasive patient care. In the setting of a rising incidence of thyroid disease, we believe our study demonstrates the need for a larger and more definitive analysis (i.e. prospective vs. randomized control trial) of the relationship between technique and non-diagnostic rates, in an attempt to minimize this inefficiency and help reduce costs associated with repeat FNAB.

Compliance with Ethical Standards

Conflict of interest There are no conflicts of interest.

Ethical Approval This article does not contain any studies with animals performed by any of the authors.

Informed Consent A minimal risk IRB was approved in conjunction with a HIPAA waiver for informed consent.

References

1. Cooper DS, Doherty GM, Haugen BR, et al. Revised American Thyroid Association management guidelines for patients with thyroid nodules and differentiated thyroid cancer. Thyroid. 2009;19:1167–214.
2. Gharib H. Fine-needle aspiration biopsy of thyroid nodules: advantages, limitations, and effect. Mayo Clin Proc. 1994;69:44–9.
3. Baloch ZW, LiVolsi VA. Fine-needle aspiration of thyroid nodules: past, present, and future. Endocr Pract. 2004;10:234–41.
4. Mazzaferri EL. Management of a solitary thyroid nodule. N Engl J Med. 1993;328:553–9.
5. Robitschek J, Straub M, Wirtz E, Klem C, Sniezek J. Diagnostic efficacy of surgeon-performed ultrasound-guided fine needle aspiration: a randomized controlled trial. Otolaryngol Head Neck Surg. 2010;142:306–9.
6. Carmeci C, Jeffrey RB, McDougall IR, Nowels KW, Weigel RJ. Ultrasound-guided fine-needle aspiration biopsy of thyroid masses. Thyroid. 1998;8:283–9.
7. Cesur M, Corapcioglu D, Bulut S, et al. Comparison of palpation-guided fine-needle aspiration biopsy to ultrasound-guided fine-needle aspiration biopsy in the evaluation of thyroid nodules. Thyroid. 2006;16:555–61.
8. Bongiovanni M, Spitale A, Faquin WC, Mazzucchelli L, Baloch ZW. The Bethesda system for reporting thyroid cytopathology: a meta-analysis. Acta Cytol. 2012;56:333–9.
9. Williams BA, Bullock MJ, Trites JR, Taylor SM, Hart RD. Rates of thyroid malignancy by FNA diagnostic category. J Otolaryngol Head Neck Surg. 2013;42:61.
10. Cibas ES, Ali SZ. The Bethesda system for reporting thyroid cytopathology. Thyroid. 2009;19:1159–65.
11. Naïm C, Karam R, Eddé D. Ultrasound-guided fine-needle aspiration biopsy of the thyroid: methods to decrease the rate of unsatisfactory biopsies in the absence of an on-site pathologist. Can Assoc Radiol J. 2013;64:220–5.
12. İnci MF, Özkan F, Yüksel M, Şalk, Şahin M. The effects of sonographic and demographic features and needle size on obtaining adequate cytological material in sonography-guided fine-needle aspiration biopsy of thyroid nodules. Endocrine. 2013;43:424–9.
13. Degirmenci B, Haktanir A, Albayrak R, et al. Sonographically guided fine-needle biopsy of thyroid nodules: the effects of nodule characteristics, sampling technique, and needle size on the adequacy of cytological material. Clin Radiol. 2007;62:798–803.
14. Brennan PA, Mackenzie N, Oeppen RS, Kulamarva G, Thomas GJ, Spedding AV. Prospective randomized clinical trial of the effect of needle size on pain, sample adequacy and accuracy in head and neck fine-needle aspiration cytology. Head Neck. 2007;29:919–22.
15. Bhakti AM, Brewer B, Robinson-Smith T, Nikiforov Y, Steward DL. Adequacy of surgeon-performed ultrasound-guided thyroid fine needle aspiration biopsy. Otolaryngol Head Neck Surg. 2008;139(1):27–31.
16. Cibas ES, Alexander EK, Benson CB, et al. Indications for thyroid FNA and pre-FNA requirements: a synopsis of the National Cancer Institute Thyroid Fine-Needle Aspiration State of the Science Conference. Diagn Cytopathol. 2008;36:390–9.
17. Wong LQ, Baloch ZW. Analysis of the bethesda system for reporting thyroid cytopathology and similar precursor thyroid cytopathology reporting schemes. Adv Anat Pathol. 2012;19:313–9.
18. Moon HJ, Kwak JY, Kim EK, Kim MJ. Ultrasonographic characteristics predictive of nondiagnostic results for fine-needle aspiration biopsies of thyroid nodules. Ultrasound Med Biol. 2011;37:549–55.

19. Tublin ME, Martin JA, Rollin LJ, Pealer K, Kurs-Lasky M, Ohori NP. Ultrasound-guided fine-needle aspiration versus fine-needle capillary sampling biopsy of thyroid nodules: does technique matter? J Ultrasound Med. 2007;26:1697–701.
20. Schoedel KE, Tublin ME, Pealer K, Ohori NP. Ultrasound-guided biopsy of the thyroid: a comparison of technique with respect to diagnostic accuracy. Diagn Cytopathol. 2008;36:787–9.
21. Tauro LF, Lobo GJ, Fernandes H, et al. A comparative study on fine needle aspiration cytology versus fine needle capillary cytology in thyroid nodules. Oman Med J. 2012;27:151–6.
22. de Carvalho GA, Paz-Filho G, Cavalcanti TC, Graf H. Adequacy and diagnostic accuracy of aspiration versus capillary fine needle thyroid biopsies. Endocr Pathol. 2009;20:204–8.
23. Dey P, Ray R. Comparison of fine needle sampling by capillary action and fine needle aspiration. Cytopathology. 1993;4:299–303.
24. Zhou JQ, Zhang JW, Zhan WW, et al. Comparison of fine-needle aspiration and fine-needle capillary sampling of thyroid nodules: a prospective study with emphasis on the influence of nodule size. Cancer Cytopathol. 2014;122:266–73.
25. Gümüş M, Cay N, Algin O, et al. Comparison of 21 and 27 gauge needles for determining sample adequacy in the aspiration biopsy of thyroid nodules. Diagn Interv Radiol. 2012;18:102–5.
26. Tangpricha V, Chen BJ, Swan NC, Sweeney AT, de las Morenas A, Safer JD. Twenty-one-gauge needles provide more cellular samples than twenty-five-gauge needles in fine-needle aspiration biopsy of the thyroid but may not provide increased diagnostic accuracy. Thyroid. 2001;11:973–6.