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Diagnostic Thyroidectomy May Be Preferable in Patients With Suspicious Ultrasonography Features After Cytopathology Diagnosis of AUS/FLUS in the Bethesda System

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Abstract: Atypia/follicular lesion of undetermined significance (AUS/FLUS) is a new category in the Bethesda System for Reporting Thyroid Cytopathology (BSRTC) for which repeat fine-needle aspiration cytology (FNAC) is recommended. The aim of this study was to identify specific ultrasonography and clinical predictors of malignancy in a subset of thyroid nodules associated with cytology diagnoses of AUS/FLUS.

Between January 2011 and December 2102, 5440 patients underwent thyroid surgery at our institution. Of these, 213 patients were diagnosed AUS/FLUS at the preoperative cytopathology diagnosis. The frequency of FNAC and ultrasonography images was compared between patients with cancerous and benign tumors based on their final pathology.

Of the 213 patients, 158 (74.2%) were diagnosed with thyroid carcinoma in their final pathology reports. In univariate and multivariate analyses, the frequency of FNAC was not significantly correlated with the cancer diagnosis. Hypoechogenicity (odds ratio 2.521, $P = 0.007$) and microcalcification (odds ratio 3.247, $P = 0.005$) were statistically correlated with cancer risk.

Although AUS/FLUS in cytopathology is recommended for repeating FNAC in BSRTC, we proposed that thyroid nodules with ultrasonography findings that suggest the possibility of cancer should undergo thyroidectomy with diagnostic intent.

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Abbreviations: AUS/FLUS = atypia or follicular lesion of undetermined significance, BSRTC = Bethesda system for reporting thyroid cytopathology, FNAC = fine-needle aspiration cytology, US = ultrasonography.

INTRODUCTION

Thyroid cancer is the first leading cancer accounting for 17.8% of all new cancers diagnosed in the Korean population.[1]

Cervical ultrasonography (US) is mandatory before surgery for thyroid cancer and is recommended for thyroid nodule evaluation. Fine-needle aspiration cytology (FNAC) under ultrasonography guidance represents the critical initial diagnostic test used to evaluate thyroid nodules and stratify the risk of malignancy. FNAC plays a key role in disease management.[2,3]

Traditionally, cytological interpretations categorize the specimen into 1 of the 4 diagnostic categories: negative, positive, nondiagnostic, and indeterminate.

As thyroid nodules are evaluated and treated, the first question the cytopathologist encounters is regarding specimen adequacy. If the cytopathologist encounters cellular paucity, most diagnose the nodules as nondiagnostic. The second question that clinicians encounter is indeterminate diagnoses. Indeterminate cytological diagnoses occur in follicular patterned thyroid lesions owing to overlapping cytomorphologic characteristics of benign and malignant lesions.[4] The distinction between benign and malignant thyroid nodules has remained challenging for clinicians.

To improve the classification, a revised classification system for reporting thyroid FNAC was proposed at the National Cancer Institute State of Science conference in 2007. This led to the Bethesda System for Reporting Thyroid Cytopathology (BSRTC), which was adopted in 2009 and comprised the following 6 classifications: unsatisfactory/nondiagnostic (category I), benign (category II), atypia or follicular lesion of undetermined significance (AUS/FLUS) (category III), follicular neoplasm/suspicious for follicular neoplasm (category IV), suspicious for malignancy (category V), and positive for malignancy (category VI) (Table 1).[5]

The traditional indeterminate group was further divided into the AUS/FLUS (category III) and follicular neoplasm (category IV) categories in BSRTC

AUS/FLUS is a new category in the BSRTC, with malignancy rates of ~5% and 15%, for which repeat FNAC is recommended.[5] According to the BSRTC, the reported rates of AUS/FLUS have been between 3% and 18% of thyroid FNAC. Furthermore, the frequency of AUS/FLUS diagnosis should be ~7% or less of all thyroid FNAC.[6–8]

As per the synopsis of the NCISSC, patients with AUS/FLUS FNAC should undergo a second FNAC after 3 months based on clinical correlations and/or ultrasound findings.[5,9–11] Performing a repeat FNAC before 3 months is hypothesized to increase the chance of reparative atypia of follicular cells,

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TABLE 1. The Bethesda System for Reporting Thyroid Cytopathology: Implied Risk of Malignancy a Recommended Clinical Management[5]

Diagnostic Category	Risk of Malignancy (%)	Usual Management
Nondiagnostic or unsatisfactory	1–4	Repeat FNAC
Benign	0–3	Clinical follow-up
Atypia of undetermined significance or follicular lesion of undetermined significance	~5–15	Repeat FNAC
Follicular neoplasm or suspicious for follicular neoplasm	15–30	Surgical lobectomy
Suspicious for malignancy	60–75	Near-total thyroidectomy or surgical lobectomy
Malignant	97–99	Near-total thyroidectomy

FNAC = fine-needle aspiration cytology.

increasing the potential for false-positive diagnoses of malignancy (Table 1).[12]

Some concerns and confusion were encountered when adapting the AUS diagnosis according to the BSRTC. First, AUS/FLUS is a heterogeneous category that contain follicular cells and/or nuclear atypia that exceed benign changes, but the changes are not enough to justify classification into any of the other diagnostic categories.[5,12,13] Thus, it has been suggested that AUS could be further subclassified into more distinct subtypes that consulting a distinct consequence for the risk of malignancy.[13–16]

Second, although patients with a repeated diagnosis of AUS/FLUS require surgical intervention,[6] the benefit for patients between repeated FNAC and surgical intervention is not yet clearly established.

The aim of this study was to identify specific ultrasonography and clinical predictors of malignancy in a subset of thyroid nodules associated with cytology diagnoses of AUS/FLUS at our institution and optimal surgical treatment of these nodules.

MATERIALS AND METHODS

Patient Cohort and Selection

This study was approved by the Institutional Review Board of Gangnam Severance Hospital, Yonsei University College of Medicine, and was conducted according to the principles of the Helsinki Declaration (IRB No.3-2013-0246).

Between January 2011 and December 2012, a total of 8244 FNAC were performed at the Thyroid Cancer Center, Gangnam Severance Hospital, Yonsei University College of Medicine, Seoul, Korea. Of these, 757 FNACs (9.2%) were interpreted as AUS/FLUS in BSRTC. Patients who underwent thyroid surgery for recurrent thyroid cancer were excluded.

Patients with typical features for thyroid cancer on ultrasonography imaging were recommended thyroidectomy, and patients with controversial ultrasonography findings who wanted repeated FNAC received repeated FNAC. If patients had controversial ultrasonography findings repeatedly, they were recommended for the same process.

Finally, of the 757 cases of AUS/FLUS, 284 cases (37.5%) that were reported as suspicious for follicular neoplasm (category IV), suspicious for malignancy (category V), or malignant (category IV) underwent thyroidectomy, 213 cases (28.1%) underwent thyroidectomy after a diagnosis of AUS/FLUS, and 260 cases (34.4%) were closely observed.

During the same period, a total of 5440 patients underwent thyroid surgery. Of these, 213 (3.9%) patients were AUS/FLUS at preoperative cytopathology diagnosis.

After identification of the enrolled patients, medical records including patient demographics, subsequent clinical outcomes, imaging studies such as ultrasonography, FNAC results, and surgical pathology results, were retrospectively reviewed. The surgical extent for thyroid cancer was decided by the American Thyroid Association (ATA) and Korean Thyroid Association (KTA) guidelines.[2,3]

Classification of Ultrasonography Findings of Nodules With AUS/FLUS

US features were described based on the ''Guidelines for Thyroid US'' by the Thyroid Study Group, Korean Society of Head and Neck Radiology.[17]

US findings of the thyroid nodules were evaluated for the following categories: shape, margins, echogenicity, and calcification. Nodule shape was described as oval-to-round, taller than wide or irregular. The margin was described as well defined, ill defined, or speculated. The presence of calcification was described as none, microcalcification (<1 mm), or macrocalcification (≥ 1 mm). The echogenicity of the nodule was described as hyper-, iso-, or hypoechogenic compared with the thyroid gland or as markedly hypoechogenic compared with the adjacent strap muscle. US criteria for malignant modules were taller-than-wide, speculated margin, marked hypoechogenicity, and the presence of micro- or macrocalcifications.[17]

FNAC and Cytology Interpretation

FNAC were performed under ultrasound guidance using 3 to 4 passes with 22-gauge needles. Syringes with FNAC were rinsed in a methanol–water solution (ThinPrep CytoLyt, Hologic).

A board-certified cytopathologist made cytology interpretations and classifications based on the BSRTC.[5] The transition to BSRTC took place in December 2009 at our institution. The BSRTC categories were comprised of the following: unsatisfactory/nondiagnostic, benign, AUS/FLUS, follicular neoplasm/suspicious for follicular neoplasm, suspicious for malignancy, and positive for malignancy (Table 1).

Statistical Analyses

Student *t* tests were used for univariate analyses when comparing selected variables. Pearson's chi squared tests were

TABLE 2. Univariate and Multivariate Analyses of Times of FNAC and Ultrasonographic Features

	Univariate Analyses			Multivariate Analyses	
Variables	**Benign (n = 55)**	**Malignant (n = 158)**	***P* Value**	**Odds Ratio (95% CI)**	***P* Value**
Times of FNAC			0.573	1.139 (0.443–2.932)	0.787
1 (n = 179)	48 (26.8%)	131 (73.2%)			
2 (n = 24)	4 (16.7%)	20 (83.3%)			
3 (n = 10)	3 (30.0%)	7 (70.0%)			
Ultrasonography features					
Taller than wide	0 (0.0%)	20 (12.7%)	0.003	–	0.998
Ill-defined margin	21 (38.2%)	92 (58.2%)	0.012	1.482 (0.746–2.943)	0.262
Hypoechogenecity	29 (52.7%)	120 (75.9%)	0.002	2.521 (1.393–5.431)	0.007
Microcalcification	9 (16.4%)	67 (42.4%)	0.001	3.247 (1.533–8.027)	0.005

CI = confidence interval, FNAC = fine-needle aspiration cytology.

used to analyze categorical and nonparametric data. Multiple logistic regression analyses with a forward stepwise variable selection procedure were used to select significant variables and identify independent predictors of malignancy. A P value of <0.05 was considered statistically significant.

RESULTS

There were 757 AUS/FLUS on preoperative FNAC. Of these, 213 cases (28.1%) underwent thyroidectomy under the preoperative diagnosis of AUS/FLUS. Of the 213 patients, 158 (74.2%) were diagnosed with thyroid carcinoma at the final pathology reports.

In univariate analyses, repeated times of FNAC did not correlate with cancer risk. Patients with repeated FNAC (>2 times) were similar to patients with single FNAC (Table 2, $P = 0.573$).

Based on US findings, thyroid nodules that were confirmed as carcinoma on the final pathology reports showed significantly more malignant features than benign confirmed nodules: taller than wide (12.7% vs 0%, $P = 0.003$), ill-defined margins (58.2% vs 38.2%, $P = 0.010$), hypoechogenicity (75.9% vs 52.7%, $P = 0.001$), and microcalcification (17.1% vs 12.7%, $P = 0.001$) (Table 2).

In multivariate analyses, the frequency of FNAC was not significantly correlated with cancer diagnosis. From the US findings, hypoechogenicity (odds ratio 2.521, $P = 0.007$) and microcalcification (odds ratio 3.247, $P = 0.005$) were significantly correlated with cancer risk (Table 2).

Scoring using variables that were statistically significant in Table 2 (taller than wide, ill-defined margin, hypoechogenicity, and microcalcification) showed that as the number of malignant US characteristics of cancer increased, the possibility of cancer is increased (Pearson's correlation coefficient = 0.990, $P < 0.001$) (Table 3).

DISCUSSION

FNAC has an essential role in the evaluation of thyroid nodules. Traditionally, the cytological interpretation categorizes the specimen into 1 of the 4 diagnostic categories: negative, positive, nondiagnostic, and indeterminate.

However, some thyroid FNACs are not easily classified into the benign, suspicious, or malignant categories. Indeterminate diagnoses have been used to represent follicular patterned thyroid lesions due to the overlapping cytomorphologic characteristics of benign and malignant lesions.

After the National Cancer Institute Thyroid Fine Needle Aspiration State of the Science Conference took place in October 2007, the Bethesda System for Reporting Thyroid Cytopathology (BSRTC) was proposed. BSRTC was adopted worldwide in 2009 and our institution adopted in 2009.

In BSRTC, some thyroid FNACs that are not easily classified into benign or malignant were reported as ''Atypia of Undetermined Significance (AUS)'' or ''Follicular Lesion of Undetermined Significance (FLUS)'' in the Bethesda System for Reporting Thyroid Cytology. AUS/FLUS are included in the indeterminate diagnoses.

The risk of malignancy for all AUS/FLUS cases including those patients with benign results at follow-up and who did not undergo surgery is presumably ~5% to 15%.[6] The malignancy rates in recently published studies using the BSRTC show a

TABLE 3. Scoring Using Variables that Have Statistical Significance in Table 2

Number of Variables	Benign (n = 55)	Malignant (n = 158)	Pearson's Correlation Coefficient (ρ)	*P* Value
0	16 (51.6%)	15 (48.4%)	0.990	<0.001
1	22 (36.1%)	39 (63.9%)		
2	14 (19.2%)	59 (80.8%)		
3	3 (7.3%)	38 (92.7%)		
4	0 (0%)	7 (100%)		

Variables: taller than wide, ill-defined margin, hypoechogenicity, and microcalcification.

wide range of 6% to 48% in resected cases and 5% to 27% in all cases.[8,18–20]

In the present study, the overall neoplastic rate for patients with at least 1 AUS/FLUS diagnosis was 9.2% (757 out of 8244) for all cases and 28.1% (213 out of 757) for cases with surgical intervention.

AUS/FLUS in the Bethesda System has been a difficult and challenging category for pathologists and clinicians, because the risk of malignancy is lower and likely closer to 5% to 15%.

For pathologists, the AUS/FLUS category should be used as a last resort and limited to ~7% or less of all thyroid FNACs. The risk of malignancy for an AUS/FLUS nodule is difficult to ascertain because only a minority of cases in this category have surgical intervention. Those that are surgically resected represent a select population of patients with repeated AUS/FLUS results or patients with worrisome clinical or US findings.[21]

For clinicians, the classification of AUS/FLUS thyroid FNAC biopsy specimens can result in difficulty in terms of clinical management and follow-up. Benign categorization is followed clinically, and follicular neoplasm/suspicious for malignancy categories are subject to surgical intervention. The AUS/FLUS category is less clearly clinically defined, and management varies from repeat FNAC (after 3–6 months) to surgical evaluation. Once AUS/FLUS has been diagnosed for a thyroid FNAC specimen, the clinician must decide on how to proceed and manage the patient.

The recommended approach for initial AUS/FLUS is to repeat FNAC (Table 1).[5] A repeat FNAC usually results in a more definitive diagnosis, and only ~20% to 25% of nodules repeatedly receive a diagnosis of AUS/FLUS.[8,22] However, there are no recommended guidelines for repeated AUS/FLUS. Some patients are recommended for repeat FNAC whereas others are recommended for diagnostic surgical resection.

Repeat FNAC were more prevalent than surgical resection. Repeat FNAC to follow up AUS/FLUS results is more cost effective than diagnostic surgical resection.[23] Moreover, clinicians cannot easily recommend surgical resection because clinicians and patients are not certain of carcinoma.

Increased efforts have focused on solving the limitations of AUS/FLUS. Subclassification of AUS/FLUS, molecular tests such as evaluation of BRAF mutation, and core-needle biopsies have been recommended and performed.[13,24] However, none of these methods solve these limitations.

US findings are the most important findings for clinicians. US findings that predict malignancy may be valid due to their high specificity and positive predictive value. In the present study, we examined some US features to evaluate their associated impacts on malignancy rates in patients with a preoperative cytological diagnosis of AUS/FLUS and subsequent surgical intervention.

Some US features, including taller than wide, ill-defined margin, hypoechogenicity, and microcalcification, were sufficient to identify malignant nodules in univariate analyses. Hypoechogenicity (odds ratio 2.521, $P = 0.007$) and microcalcification (odds ratio 3.247, $P = 0.005$) were significantly correlated with cancer (Table 2). Specifically, as the number of malignant US characteristics increased, the possibility of cancer increased (Pearson's correlation coefficient = 0.990, $P < 0.001$) (Table 3). We also investigated the correlation between FNAC times and cancer diagnosis; however, the malignancy rate did not increase according to FNAC times.

CONCLUSIONS

Repeated AUS/FLUS results in preoperative FNAC are challenging for pathologists and clinicians. If indications for thyroidectomy, regardless of AUS/FLUS results, were established, repeat biopsies do not appear necessary. Although AUS/FLUS in cytopathology is recommended for repeating FNAC in BSRTC, thyroidectomy with diagnostic intent may be recommended for thyroid nodules with US findings that suggest the possibility of cancer.

REFERENCES

1. Jung KW, Won YJ, Kong HJ, et al. Cancer statistics in Korea: incidence, mortality, survival and prevalence in 2010. *Cancer Res Treat.* 2013;45:1–14.
2. 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: 1167–1214.
3. Yi KH, Park YJ, Koong SS, et al. Revised Korean Thyroid Association management guidelines for patients with thyroid nodules and thyroid cancer. *J Korean Thyroid Assoc.* 2010;3:65–96.
4. Ravetto C, Colombo L, Dottorini ME. Usefulness of fine-needle aspiration in the diagnosis of thyroid carcinoma: a retrospective study in 37,895 patients. *Cancer.* 2000;90:357–363.
5. Cibas ES, Ali SZ. The Bethesda system for reporting thyroid cytopathology. *Thyroid.* 2009;19:1159–1165.
6. Dincer N, Blaci S, Yazgan A, et al. Follow-up of atypia and follicular lesions of undetermined significance in thyroid fine needle aspiration cytology. *Cytopathology.* 2013;24:385–390.
7. Kocjan G, Chandra A, Cross PA, et al. The interobserver reproducibility of thyroid fine-needle aspiration using the UK Royal College of Pathologists' classification system. *Am J Clin Pathol.* 2011;135: 852–859.
8. Nayar R, Ivanovic M. The indeterminate thyroid fine needle aspiration: experience from an academic center using terminology similar to that proposed in the 2007 National Cancer Institute Thyroid Fine Needle Aspiration State of the Science Conference. *Cancer.* 2009;117:195–202.
9. Faquin WC, Baloch ZW. Fine-needle aspiration of follicular patterned lesions of the thyroid: diagnosis, management, and follow-up according to national cancer institute (NCI) recommendations. *Diagn Cytopathol.* 2010;38:731–739.
10. Baloch ZW, Cibas ES, Clark DP, et al. The National Cancer Institute Thyroid fine needle aspiration state of the science conference: a summation. *Cytojournal.* 2008;5:6.
11. Layfield LJ, Abrams J, Cochand-Priollet B, et al. Post-thyroid FNA testing and treatment options: a synopsis of the national cancer institute thyroid fine needle aspiration state of the science conference. *Diagn Cytopathol.* 2008;36:442–448.
12. Baloch ZW, LiVolsi VA, Asa SL, et al. Diagnostic terminology and morphologic criteria for cytologic diagnosis of thyroid lesions: a synopsis of the national cancer institute thyroid fine needle aspiration state of the science conference. *Diagn Cytopathol.* 2008;36: 425–437.
13. Wu HH, Inman A, Cramer HM. Subclassification of ''atypia of undetermined significance'' in thyroid fine-needle aspirates. *Diagn Cytopathol.* 2014;42:23–29.
14. Renshaw AA. Subclassification of atypical cells of undetermined significance in direct smears of fine-needle aspirations of the thyroid. *Cancer (Cancer Cytopathol).* 2011;119:322–327.

15. Bongiovanni M, Krane JF, Ciba ES, et al. The atypical thyroid fine-needle aspiration: past, present, and future. *Cancer (Cancer Cytopathol).* 2012;120:73–86.
16. Singh RS, Wang HH. Eliminating the ''atypia of undetermined significance/follicular lesion of undetermined significance'' category from the Bethesda System for Reporting Thyroid Cytopathology. *Am J Clin Pathol.* 2011;136:896–902.
17. Moon WJ. Imaging diagnosis. Thyroid Study Group of Head and Neck Society of Korean Radiology. *Thyroid Imaging Diagnosis and Intervention*. 1st ed. Seoul, Korea: Ilchokak; 2008:pp. 150–183.
18. VanderLaan PA, Marqusee E, Krane JF. Clinical outcome for atypia of undetermined significance in thyroid fine needle aspirations: should repeated FNA be the preferred initial approach? *Am J Clin Pathol.* 2011;135:770–775.
19. Theoharis CG, Schofield KM, Hammers L, et al. The Bethesda thyroid fine-needle aspiration classification system: year 1 at an academic institution. *Thyroid.* 2009;19:1215–1223.
20. Layfield LJ, Morton MJ, Cramer HM, et al. Implications of the proposed thyroid fine-needle aspiration category of ''follicular lesion of undetermined significance'': a five-year multi-institutional analysis. *Diagn Cytopathol.* 2009;37:710–714.
21. Tepeoğlu M, Bilezikçi B, Bayraktar SG. A histological assessment of the Bethesda system for reporting thyroid cytopathology (2010) abnormal categories: a series of 219 consecutive cases. *Cytopathology.* 2014;25:39–44.
22. Yassa L, Cibas ES, Benson CB, et al. Long-term assessment of a multidisciplinary approach to thyroid nodule diagnostic evaluation. *Cancer.* 2007;111:508–516.
23. Heller M, Zanocco K, Zydowicz S, et al. Cost-effectiveness analysis of repeat fine-needle aspiration for thyroid biopsies read as atypia of undetermined significance. *Surgery.* 2012;152:423–430.
24. Ohori NP, Singhal R, Nikiforova MN, et al. BRAF mutation detection in indeterminate thyroid cytology specimens: underlying cytologic, molecular, and pathologic characteristics of papillary thyroid carcinoma. *Cancer Cytopathol.* 2013;121:197–205.