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*Acta Radiol* 2012 53: 168
DOI: 10.1258/ar.2011.110133

Original article

# Initially non-diagnostic ultrasound-guided fine needle aspiration cytology of thyroid nodules: value and management

**Jin Chung[1], Ji Hyun Youk[1], Jeong-Ah Kim[1], Jin Young Kwak[1], Eun-kyung Kim[1], Young Hoon Ryu[2] and Eun Ju Son[1]**

[1]Department of Radiology; [2]Department of Nuclear Medicine, Yonsei University College of Medicine, Seoul, South Korea
Correspondence to: Eun Ju Son. Email: ejsonrd@yuhs.ac

## Abstract

**Background:** Ultrasound (US)-guided fine needle aspiration cytology (FNAC) is an accurate, reliable, and simple method to identify a thyroid nodule as benign or malignant. However, non-diagnostic cytology results for thyroid nodules are a major limitation of US-guided FNAC.

**Purpose:** To investigate the incidence of thyroid cancer among cases with non-diagnostic results on FNAC and to provide suggestions for the management of thyroid nodules that are initially non-diagnostic by FNAC according to ultrasonographic findings.

**Material and Methods:** From July 2006 to December 2009, 10,317 thyroid nodules in 6684 consecutive patients underwent US-guided FNAC at our institute. Among these, 871 thyroid nodules (8.4%) were diagnosed as non-diagnostic on initial cytologic evaluation and 196 underwent a second or third FNAC. Twenty-seven thyroid nodules (18.9%) underwent surgery, while 116 thyroid nodules were cytologically confirmed as benign with no remarkable change on follow-up US were included. We retrospectively reviewed the US findings for a total of 143 thyroid nodules (123 benign nodules and 20 malignant nodules). The US features that we compared included composition, echogenicity, margin, calcifications, shape, and underlying echogenicity.

**Results:** In total, thyroid cancer was diagnosed in 20 nodules (14.0%). The size of the nodule was significantly associated with malignancy ($P < 0.05$). Most of the sonographically probable benign nodules were found to be benign (97.6%). Suspicious nodules on US were thyroid cancer in 43.2% of cases. Marked hypoechogenicity, microlobulated or irregular margin, microcalcifications, and taller-than-wide shape were significant US findings that correlated with malignancy ($P < 0.05$). The diagnostic performance of ultrasound for initially non-diagnostic thyroid nodules was as follows: sensitivity of 90.0%, specificity of 65.0%, positive predictive value of 29.5%, and negative predictive value of 97.6%.

**Conclusion:** In terms of management of thyroid nodules with non-diagnostic FNAC cytology, US evaluation is a feasible and useful method for predicting malignancy.

**Keywords:** Thyroid, fine needle aspiration cytology, sonography, thyroid cancer

Submitted March 11, 2011; accepted for publication July 30, 2011

Fine needle aspiration cytology (FNAC) of a thyroid nodule is an accurate, reliable, and minimally-invasive method to identify a thyroid nodule as benign or malignant. In addition, the use of ultrasound (US) guidance can improve diagnostic accuracy by enabling accurate biopsy of a small, non-palpable thyroid nodule with visualization of the needle within the lesion. Nowadays, the main role of FNAC in analyzing thyroid nodules is to triage patients for either surgery or conservative management with benign, suspicious, or malignant cytologic evaluation. However, a dilemma occurs in the 10–21% of patients who have cytologic findings that are non-diagnostic, and the current management of non-diagnostic thyroid nodules remains controversial (1–4). Repeat aspiration of the thyroid nodules may help to correct an initial false-negative result, and Chehade *et al.* showed a reduction in the false-negative rate from 5.2% to 1.3% after a second aspiration was performed (5). On the other hand, Shin *et al.* reported that

repeat FNAC results are still non-diagnostic in 14% of cases (6). Previous studies have reported the incidence of malignancy to be 5–37% among non-diagnostic thyroid nodules (7, 8). Current opinion suggests that non-diagnostic FNACs should be repeated; however, the algorithm for managing these non-diagnostic cases has not been established.

The aims of this study were to investigate the incidence of thyroid cancer among cases with non-diagnostic results on FNAC and to provide suggestions for the management of thyroid nodules that are initially non-diagnostic by FNAC according to ultrasonographic findings.

## Material and Methods

### *Study sample*

This study was approved by our institutional review board. Informed consent was not required for this retrospective study; however, informed consent for FNAC had been obtained from all patients prior to FNAC. From July 2006 to December 2009, 10,317 focal thyroid nodules in 6684 consecutive patients underwent US-guided FNAC at our institution. Among these, 871 thyroid nodules (8.4%) were diagnosed as non-diagnostic at initial cytologic evaluation, and 196 thyroid nodules in 190 patients were analyzed by a second or third FNAC. Second FNAC was performed for 196/871 nodules with initially non-diagnostic FNAC. Among them, 60 nodules were performed the third FNAC due to non-diagnostic results both at first and second FNACs. A repeat FNAC should be more than 90 days after a previous FNAC to avoid nuclear atypia related to aspiration (9). Surgery was performed on 27 thyroid nodules (18.9%, 27 patients), and 116 thyroid nodules were considered benign and were cytologically confirmed as benign with no remarkable change on follow-up US. In the case of benign nodules, patients had to be followed up for more than 12 months (mean 14.1 months, range 12–36 months). The remaining 53 thyroid nodules were excluded owing to a lack of final cytopathological results such as surgery or follow-up FNAC. Among them, 11 nodules were continuously non-diagnostic at first, second, and third FNACs and the remaining 42 nodules were non-diagnostic at first and second FNACs. Finally, 143 thyroid nodules (27 surgically confirmed thyroid nodules and 116 cytologically confirmed thyroid nodules with no remarkable change on follow-up US) were included in this study (Fig. 1).

This study included 143 thyroid nodules in 138 patients (118 women, 20 men). The mean age of the patients was 50.5 years (range 26–79 years), with a mean age of 49.3 years (range 27–67 years) for male patients and a mean age of 50.7 years (range 26–79 years) for female patients. The mean lesion size was 10.4 mm (range 3–40 mm). Fourteen thyroid nodules were palpable and the remaining 129 thyroid nodules (90.2%) were incidentally found on US.

### *Imaging surveillance*

Thyroid US images were obtained using a 7- to 15-MHz linear array transducer (HDI 5000; Philips Medical Systems, Bothell, WA, USA), or a 5- to 12-MHz linear array transducer (iU22; Philips Medical Systems) for the evaluation of thyroid glands and necks. Real-time US was performed by one of three radiologists (two radiologists with more than 5 years of experience in thyroid US and one radiologist with more than 2 years of experience in thyroid US).

The ultrasonographic findings of 143 thyroid nodules were retrospectively reviewed. The length, width, and depth of each nodule were evaluated on US images and the size was determined based on the longest aspect. The location (right and left) of each nodule was also reported. Focal thyroid nodules were interpreted using US features, including internal composition (solid, cyst, mixed, sponge $<50\%$, and sponge $>50\%$), echogenicity (hyper, iso, hypo, and markedly hypoehcoic), margin (circumscribed, microlobulated, and irregular), combined calcifications (micro, macro, eggshell, negative, and mixed), shape (parallel and non-parallel) and underlying echogenicity (homogeneous and heterogeneous).

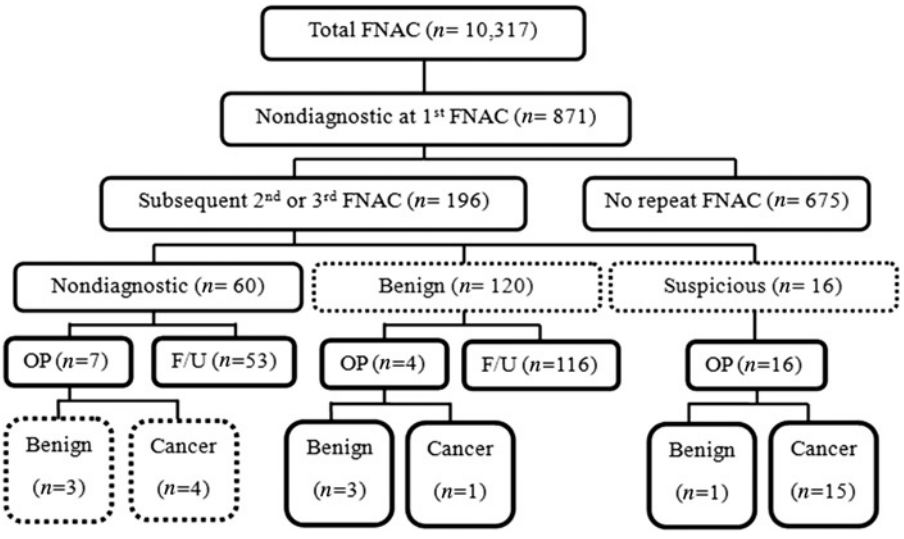


**Fig. 1** Summary of thyroid nodules characteristics. Stippled lined box represents final inclusion criteria

Findings on the final US assessment were classified as probably benign, low suspicious, or suspicious. Malignant US features were defined as marked hypoechogenicity (decreased echogenicity compared with the surrounding strap muscle), microlobulated margin (the presence of many small lobules on the surface of a nodule), or irregular margin, microcalcifications, and taller-than-wide shape (greater in the anteroposterior dimension than the transverse dimension) on the basis of previously published criteria (10). A low suspicious nodule (positive US feature) was defined if one of the above findings was present. A suspicious nodule was defined as a thyroid nodule with two or more suspicious features on US. If a nodule showed no suspicious features, it was classified as probably benign.

### *US-guided FNAC and the reference standard*

US-guided FNAC of thyroid nodule was performed by a radiologist. FNACs were performed for thyroid nodules with suspicious US features. In patients with more than one nodule, the most suspicious nodule was aspirated in accordance with the criteria mentioned above, or the largest thyroid nodule was aspirated if no suspicious US features were detected. US-guided FNAC was performed with a 23-gauge needle (Korea Vaccine, Seoul, Korea) attached to a 2-mL disposable syringe (Kovax-Syringe, Seoul, Korea) without an aspirator. Materials obtained from the aspiration were expelled onto four glass slides and smeared. All smears were immediately placed in 95% alcohol for Papanicolau and hematoxylin and eosin staining. The remainder of the materials in the syringe was rinsed in saline for processing as a cell block. Additional special staining was performed on a case-by-case basis according to the cytopathologists needs. One of three cytopathologists (over 10 years of experience) interpreted the FNAC slides.

Specimens were considered non-diagnostic if there was insufficient cellular material (<6 groups comprised of >15 well-preserved cells each), if blood was predominantly present, or if both colloid and follicular cells were present.

### *Statistical analysis*

We compared patients in the benign and malignant groups according to categorical variables by chi-square test (nominal data). We also compared continuous variables between benign and malignant groups by independent two-sample *t*-test. Diagnostic performance of US and the incidence of thyroid cancer among the initially non-diagnostic cases of FNAC were calculated. Statistical significance was assumed when the *P* value was <0.05. Ninety-five percent confidence intervals were calculated for proportions based on exact binomial tables. Statistical analysis was performed with SPSS version 15.0 software (SPSS, Chicago, IL, USA).

## Results

### *Patients and final cytopathological results*

A total of 143 thyroid nodules from 138 patients were included in our study. Of these patients, 118 (85.5%) were female and 20 (14.5%) were male. The mean size of the thyroid nodule on US was 10.4 mm (range 3–40 mm). Fourteen thyroid nodules (9.8%) were palpable and the others were incidentally found on US. Eighty-nine nodules (62.2%) were located in the right lobe of the thyroid and 54 nodules (37.8%) were located in the left lobe of the thyroid.

From a total of 143 nodules, thyroid cancer was diagnosed by surgery in 20 nodules (14.0%, 19 papillary carcinomas and one minimally-invasive Hurthel cell cancer) and the benign tumors were diagnosed in 123 nodules (7 nodules confirmed by means of surgery and 116 nodules confirmed by means of repeat FNAC and follow-up US). The patient demographics are listed in Table 1. Malignant nodules were significantly smaller than benign nodules ($P < 0.05$). Differences in age, sex, nodule location, and symptoms were statistically insignificant between benign and malignant nodules.

**Table 1** Comparison of patient demographics by final cytopathology for 143 thyroid nodules with initially non-diagnostic FNAC results

| Characteristic | Malignant | Benign | *P* value |
|---|---|---|---|
| Age (years) (mean) | 50.7 | 50.4 | 0.453 |
| Sex, *n* (%) | | | 0.275 |
| Men | 2 (8.0) | 23 (92.0) | |
| Women | 18 (15.3) | 100 (84.7) | |
| Lesion size (mm) (mean) | 8.7 | 10.7 | 0.017 |
| Location, *n* (%) | | | 0.469 |
| Right | 13 (14.6) | 76 (85.4) | |
| Left | 7 (13.0) | 47 (87.0) | |
| Symptom, *n* (%) | | | 0.675 |
| Palpable | 1 (7.1) | 13 (92.9) | |
| Incidental | 19 (14.7) | 110 (85.3) | |

### *US analysis*

The US features of thyroid nodules are summarized in Table 2. Sonographically probable benign nodules were found to be benign in 97.6% of cases. Probably benign, low suspicious, and suspicious nodules on US were found to be thyroid cancers in 2.4%, 8.3%, and 43.2% of cases, respectively ($P < 0.05$). Marked hypoechogenicity, microlobulated or irregular margin, microcalcifications, and taller-than-wide shape were significant US findings that correlated with malignancy ($P < 0.05$). The diagnostic performance of ultrasound for initially non-diagnostic thyroid nodules was determined to be as follows: sensitivity of 90.0%, specificity of 65.0%, positive predictive value of 29.5%, and negative predictive value of 97.6%.

## Discussion

US-guided FNAC of thyroid nodules is a method used worldwide to diagnose thyroid nodules. However, FNAC is limited by non-diagnostic results. Previous studies report a wide range for the incidence of malignancy in thyroid nodules with non-diagnostic cytologic results (6–8, 10). Our study showed non-diagnostic results at initial FNAC for 8.4% of thyroid nodules, and among these, 14%

**Table 2** Comparison of US features by cytopathology of 143 thyroid nodules that were initially non-diagnostic by FNAC

| US feature | Total, *n* (%) (*n* = 143) | Benign, *n* (%) (*n* = 123) | Malignant, *n* (%) (*n* = 20) | *P* value |
|---|---|---|---|---|
| Composition | | | | 0.050 |
| Solid | 89 (62.2) | 71 (79.1) | 18 (20.9) | |
| Mixed | 41 (28.7) | 39 (91.9) | 2 (8.1) | |
| <50% sponge | 3 (2.1) | 3 (2.1) | 0 (97.9) | |
| >50% sponge | 10 (7.0) | 10 ( 7.0) | 0 (93.0) | |
| Echogenicity | | | | <0.001 |
| Hyperechoic | 3 (2.1) | 3 (100) | 0 (0) | |
| Isoechoic | 36 (25.2) | 36 (100) | 0 (0) | |
| Hypoechoic | 92 (64.3) | 79 (85.9) | 13 (14.1) | |
| Markedly hypoechoic | 12 (8.4) | 5 (41.7) | 7 (58.3) | |
| Margin | | | | <0.001 |
| Circumscribed | 93 (65.0) | 89 (95.7) | 4 (4.3) | |
| Microlobulated | 34 (23.8) | 26 (76.5) | 8 (23.5) | |
| Irregular | 16 (11.2) | 8 (50.0) | 8 (50.0) | |
| Calcifications | | | | <0.001 |
| Microcalcifications | 15 (10.5) | 7 (46.7) | 8 (53.3) | |
| Macrocalcifications | 20 (14.0) | 18 (90.0) | 2 (10.0) | |
| Eggshell | 1 (0.7) | 1 (100) | 0 (0) | |
| Negative | 105 (73.4) | 96 (91.4) | 9 (8.6) | |
| Mixed | 2 (1.4) | 1 (50.0) | 1 (50.0) | |
| Shape | | | | <0.001 |
| Parallel | 106 (74.1) | 99 (93.4) | 7 (6.6) | |
| Taller-than-wide | 37 (25.9) | 24 (64.9) | 13 (35.1) | |
| Underlying echogenicity | | | | 0.69 |
| Homogeneous | 135 (94.4) | 116 (85.9) | 19 (14.1) | |
| Heterogeneous | 8 (5.6) | 7 (87.5) | 1 (12.5) | |
| US assessment | | | | <0.001 |
| Probably benign | 82 (57.3) | 80 (97.6) | 2 (2.4) | |
| Low suspicious | 24 (16.8) | 22 (91.7) | 2 (8.3) | |
| Suspicious | 37 (25.9) | 21 (56.8) | 16 (43.2) | |

were finally confirmed as malignancies. These findings are comparable to results from previous reports (range 5–37%) (6–8, 10). Therefore, determining the cytologic-clinicoradiologic correlation can be an important follow-up step for managing thyroid nodules with initially non-diagnostic cytologic results.

Previous studies have shown that the mean size of benign thyroid nodules is larger than that for malignant thyroid nodules, but the difference is not significant (10–12). More recent large-scale studies have shown increasing rates of small thyroid cancers with advances in diagnostic techniques, such as high-resolution US, and increasing numbers of imaging studies to establish the diagnostic criteria for thyroid cancer (13–15). In our study, the mean size of benign thyroid nodules was larger than that for malignant thyroid nodules, and the size of the lesion was significantly associated with malignancy (8.7 mm for malignant nodules vs. 10.7 mm for benign nodules, $P = 0.017$). However, sex, age, location, and symptoms did not differ between benign and malignant nodules. These results are probably related to selective thyroid nodules that are found incidentally on US examination. Increasing numbers of thyroid nodules are found incidentally on head and neck ultrasounds for carotid or breast disease and CT or PET scans for metastatic surveillance (16). The prevalence of incidental thyroid nodules on US has been found to be as high as 17–67% (17–19). In our study, 90.2% of thyroid nodules were found incidentally, which is the highest incidence reported to date.

Many reports have described suspicious US features, such as microlobulated or irregular margins, hypoechogenicity, microcalcifications, solid component, a taller-than-wide shape, and intratumoral vascularity (10, 20–24). In this study, we used the US classification described by Kim *et al.* because of its simplicity and high diagnostic accuracy (10). Our study also shows that the presence of microcalcifications, microlobulated or irregular margins, markedly hypoechogenicity, and taller-than-wide shape were significantly associated with malignancy.

Kwak *et al.* reported that the rate of malignancy for sonographically suspicious nodules with a non-diagnostic cytologic result was higher than the rate for probably benign nodules (60.9% vs. 7.7%, respectively) (25). Our study also showed sonographically suspicious nodules that were finally proven to be malignant in 43.2% of cases, and only 2.4% of sonographically probable benign nodules were malignant in our study. This suggests that suspicious US features are helpful in the management of thyroid nodules with initially non-diagnostic cytologic results. For thyroid nodules with non-diagnostic results by FNAC, a repeat FNAC should be required because of the high malignancy rate, which was 14.0% in this study. Repeat FNAC is needed for thyroid nodules with non-diagnostic results on initial FNAC, and correlations with US findings are helpful for the management of thyroid nodules with non-diagnostic results after the second or third FNAC. Previous studies have shown that repeat FNAC is essential for monitoring thyroid nodules with suspicious US features,

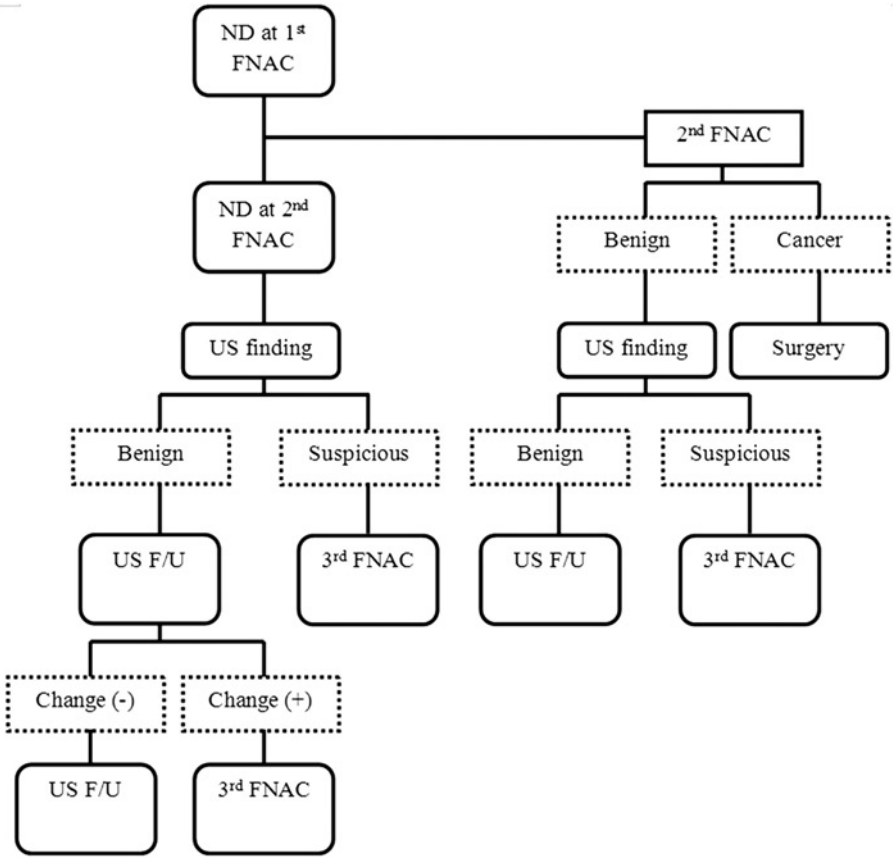


**Fig. 2** Management of thyroid nodules with non-diagnostic fine needle aspiration cytology (FNAC) results

even when the initial FNAC results are benign or indeterminate (25–27). Follow-up US is required for sonographically probable benign nodules with non-diagnostic results in FNAC, and the repeat FNAC is needed when the size or the US findings of these nodules has changed on the follow-up US (Fig. 2).

There were several limitations to our study. First, this study was a retrospective study where 81% of the lesions had not been surgically confirmed. There is a possibility that there were false-positive and false-negative cytological results. Second, the relatively small sample size ($n = 143$) could be a limitation of our study, and a study of a larger sample is needed to confirm our results. Third, three radiologists performed US-guided FNAC with interpretations of US images and thus, there may be inter-observer variability. US is a very subjective method and is highly dependent on the skill of the performer. Choi *et al.* reported that experienced radiologists (more than 6 years of experience in thyroid imaging) showed more than a moderate degree of agreement in US assessment of thyroid nodules, and their final assessments were highly accurate (28). However, our study included one inexperienced radiologist with 2 years of experience in thyroid imaging. Finally, we excluded 675 non-diagnostic nodules without repeat FNAC. Among these 675 nodules, 185 nodules had follow-up US without repeat FNAC, and the remaining 490 nodules had neither follow-up US nor repeat FNAC. These nodules had neither cytopathological results nor long-term follow-up of US; therefore, we excluded large numbers of non-diagnostic nodules according to our strict inclusion criteria. These non-diagnostic nodules without repeat FNAC might be new study materials, and we are checking for these nodules with long-term follow-up US.

In conclusion, in terms of the management of thyroid nodules with non-diagnostic FNAC cytology, US evaluation is a feasible and useful method for predicting malignancy. Repeat FNAC is essential for non-diagnostic nodules on initial FNAC, however, follow-up US is a feasible method for sonographically probable benign nodules that repeatedly have non-diagnostic results on FNAC.

**Conflict of interest:** None.

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