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Negative pressure is not necessary for using fine-needle aspiration biopsy to diagnose suspected thyroid nodules: a prospective randomized study

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Purpose: Fine-needle aspiration biopsy (FNAB) can be used to diagnose thyroid cancer and other tumors. Although FNAB without negative pressure (FNAB-P) reduces the risk of blood contamination, FNAB with negative pressure (FNAB+P) increases the sensitivity of the biopsy results. Therefore, we performed a randomized study of FNAB with or without negative pressure to identify the better diagnostic method.
Methods: Between March 2016 and February 2017, 172 consecutive patients were enrolled to investigate >0.5 cm nodules with indeterminate or suspicious malignant features. Patients were randomly assigned to the FNAB+P group (a 50 mL syringe was used to provide negative pressure) or to the FNAB-P group (passive collection of blood in the needle's hub). The 2 methods' diagnostic adequacy and quality were evaluated using an objective scoring system. The study's protocol was registered with the World Health Organization Clinical Research Information Service (http://cris.nih.go.kr/cris, KCT0001857).
Results: The patients were randomly assigned to the FNAB+P group (n = 86) or the FNAB-P group (n = 86). There were no significant intergroup differences in nodule position, size, age, consistency, calcification, *BRAF* mutation, or pathology. Evaluation of diagnostic adequacy parameters revealed no significant differences in background blood/clot (P = 0.728), amount of cellular material (P = 0.052), degree of cellular degeneration (P = 0.622), degree of cellular trauma (P = 0.979), or retention of appropriate architecture (P = 0.487). Furthermore, there was no significant intergroup difference in the diagnostic quality (P = 0.634).
Conclusion: This prospective randomized study failed to detect significant differences in the diagnostic adequacy and quality of FNAB with or without negative pressure. Therefore, the examiner may select whichever FNAB method they prefer.
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Key Words: Thyroid nodule, Fine-needle biopsy, Cytological techniques

INTRODUCTION

Fine-needle aspiration biopsy (FNAB) is useful for diagnosing thyroid cancer and other tumors, as it is a technically simple procedure with a diagnostic sensitivity of 85%–90%. This technique has higher accuracy for papillary carcinoma and lower accuracy for follicular carcinoma, relative to other thyroid tumors. However, approximately 30% of aspiration specimens are unsatisfactory and rebiopsy is recommended for those cases. Unfortunately, rebiopsies increase patient discomfort,

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as well as the time and resources needed to treat the patient, which highlights the importance of identifying the optimal FNAB method for obtaining suitable samples.

The FNAB technique can be performed without negative pressure (FNAB-P), which involves allowing the sample to passively collect in the needle's hub via capillary action. The FNAB technique can also be performed with negative pressure (FNAB+P), which involves applying negative pressure via a syringe to encourage the sample to collect in the needle's hub. The advantages of the FNAB-P approach are less blood contamination, cell degeneration, and trauma, although it may provide a relatively low number of cells. In contrast, FNAB+P ensures sufficient cell acquisition but can cause contamination with blood and other fluids [1,2].

Some studies have compared these 2 methods to determine which is more accurate and adequate for obtaining samples, with some results indicating that FNAB-P provided better sampling accuracy than FNAB+P [3-6]. However, other studies failed to detect a significant difference in sampling accuracy [7-9]. Interestingly, the previous studies have involved performing both techniques on the same mass in the same patient, which could compromise the findings of the second test if the first test caused bleeding. Moreover, most of the previous studies regarding FNAB+P have used retrospective or nonrandomized designs. Therefore, we performed a prospective randomized study to compare FNAB-P and FNAB+P for diagnosing suspicious thyroid masses. Objective indicators that were designed by Mair et al. [10] were used to determine whether one method was superior to the other.

METHODS

Study design

Between March 2016 and February 2017, 172 consecutive patients were enrolled at Daejeon St. Mary's Hospital before undergoing FNAB for suspected thyroid nodules. The randomization was performed using a randomization table, with 86 patients assigned to the FNAB+P group and 86 patients assigned to the FNAB-P group. All patients provided written informed consent before being enrolled in the study (Fig. 1). The study's protocol was approved by the Catholic University Hospital Institutional Review Board (Daejeon, Korea; DC15EISI0126) and was registered with the WHO Clinical Research Information Service (http://cris.nih.go.kr/cris, KCT0001857).

Study criteria

The study's inclusion criteria were age of >19 years, suspicious solid or mixed cystic-solid nodules with a diameter of ≥0.5 cm on the ultrasonogram, indeterminate and suspicious malignant nodules, and the provision of informed consent to participate in the study. The exclusion criteria were nodules with a diameter of <0.5 cm, purely cystic nodules, apparently benign nodules, nodules that would be difficult to access because of the surrounding blood vessels, and rebiopsy that was being performed <3 months after a previous examination.

FNAB methods (FNAB-P *vs.* FNAB+P)

The FNAB technique was performed either with or without negative pressure according to the patients' group assignments. All tests were performed under ultrasonographic guidance by a single surgeon, who could not be blinded to the patients'

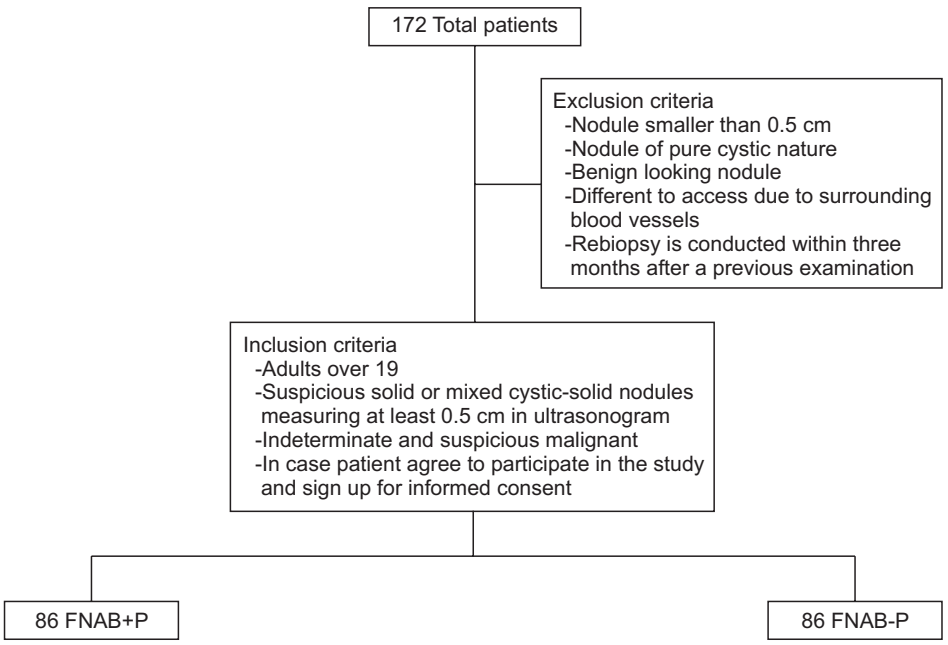

Fig. 1. Study flow chart. FNAB, fine-needle aspiration biopsy; FNAB+P, FNAB with negative pressure; FNAB-P, FNAB without negative pressure.

assignments. The FNAB+P approach was performed using a 50-mL syringe, a 10-mL syringe, a 23-G needle, and an extension tube (Fig. 2). The needle was inserted to the center of the mass under ultrasonographic guidance. The plunger from the 10-mL syringe was then used to fix the 50-mL plunger. Then negative pressure is applied to the needle through the extension line. The negative pressure was subsequently released when the sample became visible in the needle's hub. The FNAB-P approach was performed by simply inserting the needle to the center of the mass and waiting for the sample to collect in the needle's hub via capillary action. Four pathologists, who were blinded to the patients' assignments, read the pathological results and evaluated the techniques' diagnostic adequacy and quality. The evaluations were performed using a multihead microscope and the final decision was reached via consensus.

Objective scoring system

Because the test results can be subjective, an objective scoring system was applied to the slides for each sample. The system was developed by Mair et al. [10] and included background blood, amount of cellular material, degree of cellular degeneration, degree of cellular trauma, and retention of appropriate architecture (Table 1). The scores for each item were added together, and the diagnostic quality was subsequently classified as unsuitable, adequate, or superior. Unsuitable is score 0–2, adequate is score 3–6 and superior is score 7–10.

Statistical analysis

The aim of this clinical study is to investigate the diagnostic accuracy of FNAB-P and FNAB+P during cytologic examination, which is a different method for thyroid nodule cytology. In other words, we test the hypothesis that the accuracy of the two diagnostic methods is different. The proportions of each arm were randomized 1:1 and the primary endpoint was defined as the ratio of diagnostically superior (DS) or diagnostically adequate (DA) as a result of thyroid cytology. Previous studies reported a DS or DA ratio of 76%–93% in the experimental group without aspiration during thyroid cytology and 66%–86% of the expected DS or DA in the control group [1,11]. Therefore, the DS or DA ratio of the experimental group (FNAB-P) was 78% and the DS or DA ratio of the control group (FNAB+P) was 68%. We considered the primary parameter difference to be 20% meaningful. For calculating the number of samples, significant

Fig. 2. A schematic figure of fine-needle aspiration biopsy with negative pressure. A 50-mL syringe is connected to the line and cells are removed by applying negative pressure to the syringe.

Table 1. The scoring system for the pathology findings

Criterion	Qualitative description	Score
Background blood/clot	Larage amount/great compromise to diagnosis	0
	Moderate/diagnosis possible	1
	Minimal/diagnosis easy; specimen of textbook quality	2
Amout of cellular material	Minimal to abscent/diagnosis not possible	0
	Sufficient for diagnosis	1
	Abundant/diagnosis simple	2
Degree of cellular degeneration	Marked/diagnosis impossible	0
	Moderate/diagnosis possible	1
	Minimal/good preservation; diagnosis easy	2
Degree of cellular trauma	Marked; diagnosis impossible	0
	Moderate; diagnosis possible	1
	Minimal; diagnosis easy	2
Retention of appropriate architecture	Minimal to abscent/nondiagnostic	0
	Moderate/some preservation	1
	Excellent architecture display, closely reflecting histology	2
Total		10

level α was 0.05 and the power 1-β was 80%.

$$H_0 : P_c - P_t = 0.20 \text{ vs. } H_1 : P_c - P_t \neq 0.20$$

n = number of experimental group and control group

P_c = DS or DA ratio of experimental group (FNAB-P)

P_t = DS or DA ratio of the control group (FNAB+P)

$\overline{P}$ = The difference between the ratio of 2 groups = 0.20

$$\overline{Q} = 1 - \overline{P},\ Q_c = 1 - P_c,\ Q_t = 1 - P_t,\ \Delta_A^2 = (P_c - P_t)^2$$

$$n = \frac{\left(Z_{\alpha/2}\sqrt{2\overline{P}\overline{Q}} + Z_{\beta}\sqrt{P_cQ_c + P_tQ_t}\right)^2}{\Delta_A^2}$$

The number of patients required for each group is 77.05, and considering the number of people who are eliminated or excluded, about 10% (77.05/1–0.1 = 85.62) Therefore, the number of patients required for each group is 86 people, total 172 patients.

All data were analyzed using IBM SPSS Statistics ver. 22.0 (IBM Co., Armonk, NY, USA). Categorical variables were compared using the chi-square test and continuous variables were compared using Student t-test.

RESULTS

Clinical characteristics

The 172 patients included 33 men (19.2%) and 139 women (80.8%). Comparison of the FNAB+P and FNAB-P groups revealed no significant difference in mass size (P = 0.295), location distribution (right, left, and isthmus), mass consistency, mass calcification, or levels of thyroid stimulating hormone, free T4, and T3. The pathological results were nondiagnostic (n = 43, 25.0%), benign (n = 81, 47.1%), atypia of undetermined significance/follicular lesion of undetermined significance (AUS/FLUS) (n = 25, 14.53%), and malignancy (n = 23, 13.37%). Similarly, the FNAB results in the FNAP+P and FNAB-P groups were nondiagnostic (22.1% [n = 19] *vs.* 27.9% [n = 24]), benign (48.8% [n = 42] *vs.* 45.3% [n = 39]), AUS/FLUS (14.0% [n = 12] *vs.* 15.1% [n = 10]), and malignant (15.1% [n = 13] *vs.* 11.6% [n = 10]) (P = 0.774). There was no significant difference between the 2 groups in their *BRAF* mutation rates (P = 0.153) (Table 2).

Table 2. Clinical characteristics of patients who underwent fine-need aspiration biopsy with or without negative pressure

Variable	FNAB+P	FNAB-P	P-value
Age (yr), median (range)	57 (21–81)	56 (29–83)	0.848
Age (yr)			>0.999
<45	13 (15.1)	14 (16.3)	
≥45	73 (84.9)	72 (83.7)	
Tumor size (cm), median (range)	1.35 (0.5–10.4)	1.35 (0.5–5.5)	0.295
Position			0.459
Right	46 (53.5)	39 (45.3)	
Left	38 (44.2)	46 (53.5)	
Isthmus	2 (1.5)	1 (1.2)	
Confirmed diagnosis (Bethesda system)			0.774
I. Nondiagnostic	19 (22.1)	24 (27.9)	
II. Benign	42 (48.8)	39 (45.3)	
III. AUS/FLUS	12 (14)	13 (15.1)	
IV. Suspicious for follicular neoplasm	0 (0)	0 (0)	
V/VI. Suspicious for malignancy/malignancy	13 (15.1)	10 (11.6)	
BRAF mutation			0.153
Negative	50 (84.7)	56 (93.3)	
Positive	9 (15.3)	4 (6.7)	
Consistency			0.820
Cystic and solid	10 (11.6)	12 (14.0)	
Solid	76 (88.4)	74 (86.0)	
Calcification			0.199
No	52 (60.5)	61 (70.9)	
Yes	34 (39.5)	25 (29.1)	
T3 (ng/dL), mean (range)	1.66 (1.07–2.5)	1.67 (1.04–2.39)	0.113
freeT4 (ng/dL), mean (range)	1.22 (0.87–1.78)	1.26 (0.81–2.2)	0.547
TSH (μIU/mL), mean (range)	1.90 (0.05–6.13)	1.85 (0.01–6.18)	0.360

Values are presented as number (%) unless otherwise indicated.
FNAB, fine-needle aspiration biopsy; FNAB+P, FNAB with negative pressure; FNAB-P, FNAB without negative pressure; AUS/FLUS, atypia of undetermined significance/follicular lesion of undetermined significance; TSH, thyroid stimulating hormone.

Table 3. Comparing the outcomes between the group using the scoring system criteria

Criterion	FNAB+P	FNAB-P	P-value
Background blood/clot			0.728
0	15 (18.5)	14 (16.9)	
1	10 (12.3)	14 (16.9)	
2	56 (69.1)	55 (66.3)	
Amount of cellular material			0.052
0	33 (40.7)	29 (34.9)	
1	25 (30.9)	40 (48.2)	
2	23 (28.4)	14 (16.9)	
Degree of cellular degeneration			0.622
0	21 (25.9)	22 (26.5)	
1	28 (34.6)	23 (27.7)	
2	32 (39.5)	38 (45.8)	
Degree of cellular trauma			0.979
0	19 (23.5)	18 (21.7)	
1	24 (29.6)	25 (30.1)	
2	38 (46.9)	40 (48.2)	
Retention of appropriate architecture			0.487
0	33 (40.7)	33 (39.8)	
1	28 (34.6)	35 (42.2)	
2	20 (24.7)	15 (18.1)	
Total score, mean (range)	5.21 (0–10)	5.34 (0–10)	0.238

Values are presented as number (%) unless otherwise indicated. FNAB, fine-needle aspiration biopsy; FNAB+P, FNAB with negative pressure; FNAB-P, FNAB without negative pressure.

Table 4. Comparing the diagnostic performances of fine-need aspiration biopsy with or without negative pressure

	FNAB+P	FNAB-P	P-value
Diagnostic quality			0.634
Unsuitable, score 0–2	22 (25.6)	18 (20.9)	
Adequate, score 3–6	33 (38.4)	39 (45.3)	
Superior, score 7–10	31 (36.0)	29 (33.7)	

Values are presented as number (%).

Diagnostic adequacy and quality

The scoring system criteria revealed no significant intergroup differences in background blood/clot (P = 0.728), amount of cellular material (P = 0.052), degree of cellular degeneration (P = 0.622), degree of cellular trauma (P = 0.979), and retention of appropriate architecture (P = 0.487) (Table 3). The diagnostic qualities in the FNAB-P and FNAB+P groups were unsuitable (25.6% *vs.* 20.9%), adequate (38.4% *vs.* 45.3%), and superior (36.0% *vs.* 33.7%) (P = 0.634) (Table 4).

DISCUSSION

Previous studies have evaluated various techniques to confirm that FNAB is useful for diagnosing thyroid cancer and other tumors. However, most previous studies have involved retrospective protocols, and even the prospective studies were limited by the use of both techniques on the same mass, which could limit the accuracy of the FNAB results. Thus, the present study used a prospective randomized approach to ensure that each mass was only evaluated using one technique, which helps address some of the previous studies' limitations. For example, when FNAB+P and FNAB-P have previously been used for the same mass, there were inconsistencies in the findings regarding whether FNAB+P was superior or inferior to FNAB-P. This could be related to contamination of the second test if bleeding was caused during the first test, which would result in poor accuracy. The present study failed to detect significant differences in bleeding and cellular shape, although FNAP+P was associated with non-significantly higher number of cells obtained (P = 0.052). In contrast, previous studies have indicated that a greater number of cells are obtained via FNAB+P. A larger sample of patients may be needed to address this potential discrepancy.

Several methods can be used to compare the results of aspiration tests, with the ratio of diagnostic and nondiagnostic results often being used to assess their adequacy, or the test results being compared to postoperative pathology findings [7,12-14]. However, we elected to not compare the test results to postoperative pathology findings, as relatively few patients had malignancy and the study was not designed to evaluate the test's accuracy. In this study, diagnostically adequacy and quality were evaluated using a previously developed scoring system, which is a reliable and objective method.

The rates of non-diagnostic results were 22.1% for FNAP+P and 27.9% for FNAB-P, and the rates of unsuitable results were 25.6% for FNAB+P and 20.9% for FNAB-P. Similarly, previous studies have shown that FNAB-P tends to provide fewer unsuitable results than FNAB+P, with reported unsuitable rates ranging from 5.3% [15] to 43.1% [12], which indicate that our rates are not excessively high. Another study showed there was a significant effect of reducing inadequate or unsatisfactory specimen 3-pass 25-G needle compared to 1-pass 22-G needle. But there is not statistically significant compared to 2-pass 22-G needle [16]. Our study is 1 or 2 passes using 23-G needle. We stopped our aspiration biopsy when we thought we had enough tissue by grossly on the slide. We will make effort to improve the quality of the biopsy technique in the future.

Interestingly, we detected a discrepancy in the rates of unsuitable results when we compared the aspiration cytology results (FNAB+P, 22.1%; FNAB-P, 27.9%) to the scoring system results (FNAB+P, 25.6%; FNAB-P, 20.9%). In this context, the most common scores were 2 for the FNAB+P group and 0 for the FNAB-P group. However, a score of 2 might not be included in the nondiagnostic aspiration cytopathology results, which

could lower the nondiagnostic rate for the FNAB+P group. In contrast, the scoring system assigns each score a unique classification (i.e., a score of 2 indicates an unsuitable result), which would increase the unsuitable rate of the FNAB+P group in the scoring system relative to the aspiration cytopathology results.

Another unique aspect of the present study is that the negative pressure was created using an extension line and separate syringe, rather than using free handling or a syringe pistol. Although free handling has the advantage of not requiring additional instruments, it can be difficult to apply a specific amount of pressure. A syringe pistol can easily provide a pressure, although the needle tip can be inadvertently moved while manipulating the pistol. Furthermore, because the ultrasonographic guidance is lost when the pressure is applied with the other hand, which can also result in inadvertent movement of the needle tip. Thus, in the present study, negative pressure was applied using an extension tube and a 50-mL syringe by assistant, which allowed the operator to continue ultrasonographic guidance and prevent needle tip movement during aspiration of the sample.

The present study's findings are limited by the fact that the FNAB results were not compared to postoperative pathology results, although this is related to the relatively low proportion of malignancies and surgery not being performed for most benign cases. Thus, diagnostic sensitivity and specificity should likely be verified in a more comprehensive study that includes postoperative pathology findings. Nevertheless, the present study involved FNAB procedures that were performed by a single surgeon, which eliminated any interobserver variability, and the diagnostic adequacy and quality were determined via consensus between four blinded pathologists, which may make our findings more objective.

In conclusion, this prospective randomized study failed to reveal any significant differences in diagnostic adequacy and quality between thyroid FNAB with or without negative pressure. Therefore, it appears that the examiner may select whichever FNAB technique they prefer.

CONFLICTS OF INTEREST

No potential conflict of interest relevant to this article was reported.

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