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

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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.
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’
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.](image)

| Criteria                                      | Qualitative description                                                                 | Score |
|-----------------------------------------------|-----------------------------------------------------------------------------------------|-------|
| Background blood/clot                         | Large amount/great compromise to diagnosis                                              | 0     |
|                                               | Moderate/diagnosis possible                                                             | 1     |
|                                               | Minimal/diagnosis easy; specimen of textbook quality                                     | 2     |
| Amount of cellular material                   | Minimal to absent/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 absent/nondiagnostic                                                         | 0     |
|                                               | Moderate/some preservation                                                              | 1     |
|                                               | Excellent architecture display, closely reflecting histology                              | 2     |
| Total                                         |                                                                                         | 10    |
level $\alpha$ was 0.05 and the power $1-\beta$ 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)

$\Delta = $ The difference between the ratio of 2 groups = 0.20

$$2 \left( \frac{Z_{\alpha / 2} \sqrt{n} + Z_{\beta} \sqrt{n} \times P \times Q} {\Delta^2} \right)^2$$

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) ($n = 25, 14.5%$), and malignancy ($n = 23, 13.37$%). Similarly, the FNAB results in the FNAP+P and FNAP-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 ($P = 0.159$) (Table 2).

| 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 (μU/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.
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).

| 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.

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 FNAP+P and 20.9% for FNAB-P. Similarly, previous studies have shown that FNAB-P tends to provide fewer unsuitable results than FNAP+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 (FNAP+P, 22.1%; FNAB-P, 27.9%) to the scoring system results (FNAP+P, 25.6%; FNAB-P, 20.9%). In this context, the most common scores were 2 for the FNAP+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

| Diagnostic quality   | FNAP+P | FNAB-P | P-value |
|----------------------|--------|--------|---------|
| 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 (%).
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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