Comparison of cytological adequacy and pain scale score in ultrasound-guided fine-needle aspiration of solid thyroid nodules for liquid-based cytology with with 23- and 25-gauge needles: a single-center prospective study

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In ultrasound (US)-guided fine-needle aspiration (FNA) of solid thyroid nodules (STN) using liquid-based cytology (LBC), the most appropriate needle size for LBC remains unclear. This study compared the cytological adequacy and complications associated with using 23- and 25-gauge needles in US-guided FNA of STNs using LBC. US-guided FNA was performed in consecutive patients by one radiologist to diagnose STNs ≥ 5 mm in the largest diameter. The one-sampling technique through a single needle puncture and multiple to-and-fro needle motions was used in each patient. The 23- and 25-gauge needles were used consecutively each day. After FNA, the pain and complications experienced by each patient were investigated by a nurse, who was blinded to the information of needle gauge used. A cytopathologist retrospectively analyzed the cytological adequacy and cellularity of the cases. Of the 99 STNs, eight (8.1%) exhibited inadequate cytology (4 each with 23- and 25-gauge needles). The rate of cytological adequacy was not statistically different between the groups (p = 0.631). The mean pain scale values with 23- and 25-gauge needles were 2.1 ± 1.3 and 1.6 ± 1.3, respectively (p = 0.135). There were no significant complications in either group. In conclusion, both 23- and 25-gauge needles are useful in LBC because cytological adequacy and complications were not statistically different with both sizes of the needles.

Ultrasonography (US)-guided fine-needle aspiration (FNA) is a cost-effective modality used as the mainstay for initial evaluation of thyroid nodules¹. For cytological diagnosis of US-guided FNA of thyroid nodules, conventional smears, which are prepared by coating the aspirated material evenly onto multiple microscope slides, have been used for a long time⁴. Liquid-based cytology (LBC) is rapidly replacing conventional smears since its introduction in 1996⁵. LBC employs advanced concentration techniques to yield a single slide that contains most, or all, of the FNA material⁶. Numerous factors can influence the cytological adequacy of US-guided FNA of thyroid nodules using LBC: the solidity or vascularity of the thyroid nodule, sampling technique used (aspiration and capillary acquisition) and number of samples taken (single versus multiple), number of needle passes, use of aspiration devices, and gauge of the needle used⁷-⁹. In clinical practice, needles between 21 to 27 gauge are usually selected according to the institution and physician¹⁰. While one previous study demonstrated that
when solution-based samples are used, larger (21-gauge) needles provide more diagnostic specimen 7, another study demonstrated that there was no significant difference between 21-gauge and 23-gauge needles11. Hence, the most appropriate needle size to be used for LBC remains unclear. This study aimed at comparing the cytological adequacy, pain scale, and other complications associated with using 23- and 25-gauge needles in US-guided FNA of thyroid nodules using LBC.

 Patients and Methods

 Patients. This prospective study was approved by the Busan Paik Hospital Institutional Review Board. All research was performed in accordance with relevant guidelines, and informed written consent was obtained from all patients before the US-guided FNA was performed. Between January and April 2018, one radiologist, with seven years of experience in US-guided FNA of thyroid nodules, performed US-guided FNA in 120 consecutive patients (94 female, 26 male; mean age, 51.2 ± 12.5 years; range, 24 to 77 years) to diagnose thyroid nodules that were ≥5 mm in the largest diameter (mean size, 15.2 ± 10.8 mm; range, 3.0 to 60.0 mm). Among these, purely calcified thyroid nodules (n = 5), thyroid cysts (n = 8), and partially cystic thyroid nodules (n = 8) were excluded. Ninety-nine patients (79 women, 20 men; mean age, 51.5 ± 12.6 years; range, 24 to 76 years) with solid thyroid nodules (defined as thyroid nodules with a solid component accounting for ≥90% of the total volume) were included.

 Ultrasound-guided fine-needle aspiration and pain scoring. One radiologist performed US-guided FNA in all patients under the guidance of a high-resolution US modality (IU 22; Philips Medical Systems, Bothell, WA, USA) equipped with a 5- to 12-MHz linear-array transducer. The operator used a conventional 23-gauge (2.5 cm in length) or a 25-gauge (3.8 cm in length) needle attached to an empty 3-mL plastic syringe (Fig. 1). For randomization of needle-size selection, the needle gauge was changed every other day. To obtain the specimen, the one-sampling technique including a single needle puncture and multiple to-and-fro needle motions was used, without applying an aspiration device nor local anesthesia12. To obtain adequate specimens, the mixed sampling technique (i.e., initial capillary sampling followed by progressive aspiration sampling depending on the amount of aspirate to be obtained) was used12. Aspirated material was collected in CytoLyt™ solution (Hologic-Cytyc Co.) as follows: after separating the plastic syringe and needle, the contents within the syringe were injected into the LBC bottle, and then, the contents within the needle were flushed into the LBC bottle.

 A post-procedure survey assessing pain and other complications after US-guided FNA had been routinely performed in our hospital. Immediately after completing the procedure, the patient was transferred to another room. Blinded to the needle size, a nurse asked the patient to grade the pain experienced during US-guided FNA using a pain scale with a numeric pain score ranging from 0 to 10 (0 represented "no pain"; 10 represented "the worst pain"). The nurse recorded the pain score mentioned by the patients.

 Liquid-based cytology and cytological analysis. After the CytoLyt™ solution containing the aspirates was centrifuged, the obtained pellet was transferred to the PreservCyt™ (Hologic-Cytyc Co.) solution and allowed to rest for 15 minutes. In each case, one Papanicolaou-stained slide was prepared by using ThinPrep 5000 (Hologic-Cytyc Co.).

 All cytological slides were retrospectively analyzed by a cytopathologist with 16 years of experience in cytological analysis of thyroid FNA, with blinded to the data on the needle size. Specimen adequacy was determined according to the Bethesda System for Reporting Thyroid Cytopathology: six groups of ten well-visualized follicular cells was the minimal criteria13,14. Specimens that contained abundant colloid or numerous inflammatory cells were classified as adequate, even though six groups of follicular cells were not identified. On the contrary, specimens that only consisted of cystic contents were considered inadequate. To determine the cellularity, the numbers of cell clusters ≥ 10 well-visualized follicular cells were counted using a ×100 microscope in the highest cellular area. The cytopathologist classified the degree of cellularity as follows: high (more than 10 clusters),
more than 8. Intrathyroidal hemorrhage and other complications were not found in any case.  

The pain scale scores of the two groups is compared in Table 2. The mean pain scale value in the 23- and 25-gauge needle groups were 1.3 (range, 0 to 7) and 1.6 (range, 0 to 6), respectively. The 23-gauge needle group showed a higher prevalence in the high cellularity class; the 25-gauge needle group showed a higher prevalence in the intermediate cellularity class; and both the groups had similar prevalence in the low cellularity class. Cytomorphologic features were reviewed and categorized using the Bethesda classification.

**Statistical analysis.** The data was tested for normal distribution using the Kolmogorov-Smirnov test. Age at the time of diagnosis, and the size of nodule were expressed as the mean ± SD. The cases were divided into two groups based on the size of needle used (23-gauge needle group versus 25-gauge needle group). Mean differences in age and size of nodule between the two groups were compared using the independent t-test. Group comparisons of categorical variables were performed using the χ² test, and those for small cell values using the Fisher’s exact test. All statistical analyses were performed with a statistical software (SPSS, version 24.0, SPSS, Chicago, IL, USA), and P values < 0.05 were considered statistically significant.

**Ethics approval and consent to participate.** The study design was approved by the appropriate ethics review boards (IRB 18-0135), and informed written consent was obtained from all patients before the US-guided FNA was performed.

**Consent for publication.** Consent for publication was not obtained from the patient owing to patient anonymity and no patient details within the manuscript. All the authors have taken public responsibility for the manuscript content and publication.

**Results**

The mean of the largest diameters of the 99 STNs was 14.4 ± 10.5 mm (range, 5.0 to 60.0 mm). The locations of the STNs included the right lobe (n = 48), left lobe (n = 48), and isthmus (n = 1). US-guided FNA was performed using 23-gauge needle for 49 (49.5%) STNs and 25-gauge needles for 50 (50.5%) STNs. The demographic characteristics of the two groups are presented in Table 1. The sizes of the STNs were higher in the 25-gauge group (18.0 ± 11.9 mm) than in the 23-gauge group (10.7 ± 7.4 mm) (p < 0.0001). However, there were no significant between-group differences in the patient age, gender, and location of STNs (p > 0.05).

Of the 99 STNs, eight (8.1%) showed inadequate cytology: six revealed no follicular cells, whereas two showed at least one follicular cluster with 20 to 60 follicular cells. The cytological adequacy and cellularity of the two groups are compared in Table 2. The rates of cytological adequacy in the 23- and 25-gauge needle groups were 91.8% (45/49) and 92% (46/50), respectively. This difference was not statistically significant (p = 0.631). The 25-gauge needle group showed a higher prevalence in the high cellularity class; the 23-gauge needle group showed a higher prevalence in the intermediate cellularity class; and both the groups had similar prevalence in the low cellularity class. The differences in the cytological cellularity between the groups were not statistically significant (p = 0.185). Among the 99 STNs, there were Bethesda category I (n = 8), II (n = 38), III (n = 25), IV (n = 6), V (n = 11), and VI (n = 11). Bethesda category II STNs were most common in both groups. There was no significant difference in the Bethesda classification between the two groups (p = 0.711).

The pain scale scores of the two groups are compared in Table 2. The mean pain scale values in the 23- and 25-gauge needle groups were 1.3 (range, 0 to 7) and 1.6 (range, 0 to 6), respectively. The 23-gauge needle group exhibited a higher score than the 25-gauge needle group. However, the difference in the scores between the two groups was not significant (p = 0.135). Scores of 1 or 2 were predominant, and no case reported a score more than 8. Intrathyroidal hemorrhage and other complications were not found in any case.

**Discussion**

In US-guided FNA of thyroid nodules for conventional smears, thinner needles are associated with better cytological adequacy than larger needles9,15. However, for LBC, the relationship between the needle gauge used and

| Items          | 23-gauge (n = 49) | 25-gauge (n = 50) | p value |
|----------------|------------------|------------------|---------|
| Age (yr)       | 52.3 ± 11.6      | 50.7 ± 13.7      | 0.551   |
| range          | 25–76            | 24–75            |         |
| Gender         |                  |                  | 0.326   |
| female         | 37 (46.8)        | 42 (53.2)        |         |
| male           | 12 (60)          | 8 (40)           |         |
| Location of STNs |                 |                  | 0.269   |
| right          | 28 (56)          | 22 (44)          |         |
| left           | 21 (43.8)        | 27 (56.3)        |         |
| isthmus        | 0                | 1 (100)          |         |
| Size of STNs (mm) |              |                  | <0.0001 |
| mean           | 10.7 ± 7.4       | 18.0 ± 11.9      |         |
| range          | 5–39.8           | 5–60             |         |

Table 1. Comparison of demographic characteristics of the patient population according to the needle size in ultrasonography-guided fine-needle aspiration. Note. —Data are number of items, with percentage in parentheses. STN, solid thyroid nodule; US, ultrasonography.
the adequacy of the FNA specimen is still unclear. Although larger needles provide more specimen for diagnosis, the difference in cytological adequacies between 21-gauge and 23-gauge needle groups is not significant. Furthermore, the number of studies available is small. In the present study, we attempted to compare the difference in cytological adequacy of US-guided FNA of STNs for LBC between 23- and 25-gauge needles. There was no statistically significant difference. This result was similar to that of our previous study that compared 21-gauge and 23-gauge needles. Moreover, the rates of cytological adequacy when using either 23- or 25-gauge needles were high (91.8% and 92%). Thus, we believe that the use of needles between 21- and 25-gauge does not affect the cytological adequacy of US-guided FNA of STNs for LBC.

In a previous study, samples collected using 21-gauge needles in US-guided FNA of thyroid nodules for LBC produced an average cellularity of 2.18, whereas those collected using 25-gauge needles produced an average cellularity of 1.92. Although this difference was not statistically significant, the data trended toward suggesting that the use of 21-gauge needles for FNA increased the specimen cellularity (p = 0.08). Other investigators also reported that FNAs of thyroid nodules for LBC performed with 21-gauge needles led to more cellular specimens than those performed with 25-gauge needles. However, the increased cellularity from the biopsies performed with the 21-gauge needles did not increase the sufficiency rate. In our previous study, there was no significant difference in the cytological cellularity between 21- and 23-gauge needle groups. However, the 23-gauge needle group showed higher cellularity when compared with the 21-gauge needle group. The reason for the disparity between our studies and others is not clear. We did not compare the difference in cytological cellularity between using 21- and 25-gauge needles.

A disadvantage of using larger needles is that they could result in more patient discomfort. To the best of our knowledge, only one comparison study on US-guided FNA of STNs for LBC used the pain scale. In that study using the one sampling technique, the mean pain score in the 21-gauge needle group (1.4 ± 1.1) was found to be lower than that in the 21-gauge needle group (1.8 ± 1.3), although this difference was not statistically significant. In our study, although the pain score in the 23-gauge needle group (2.1 ± 1.3) was higher than that in the 25-gauge needle group (1.6 ± 1.3), the difference was not statistically significant. The reason for higher pain scores in the 23-gauge needle group may be associated with the smaller needle sizes in this group than those in the 25-gauge needle group. Thus, we believe that when using the one sampling technique in US-guided FNA of STNs, patient pain does not differ in the use of needles between 21- and 25-gauge.

| Items                     | 23-gauge (n = 49) | 25-gauge (n = 50) | p value |
|---------------------------|------------------|------------------|---------|
| Cytological adequacy      |                  |                  | 0.631   |
| adequate                  | 45 (49.5)        | 46 (50.5)        |         |
| inadequate                | 4 (50)           | 4 (50)           |         |
| Cytological cellularity   |                  |                  | 0.185   |
| low                       | 27 (49.1)        | 28 (50.9)        |         |
| intermediate              | 12 (70.6)        | 5 (29.4)         |         |
| high                      | 10 (37)          | 17 (63)          |         |
| Bethesda category         |                  |                  | 0.711   |
| I                         | 4 (50)           | 4 (50)           |         |
| II                        | 20 (52.6)        | 18 (47.4)        |         |
| III                       | 9 (36)           | 16 (64)          |         |
| IV                        | 3 (50)           | 3 (50)           |         |
| V                         | 7 (63.6)         | 4 (36.4)         |         |
| VI                        | 6 (54.5)         | 5 (45.5)         |         |
| Pain scale                |                  |                  | 0.135   |
| 0                         | 3 (23.1)         | 10 (76.9)        |         |
| 1                         | 12 (44.4)        | 15 (55.6)        |         |
| 2                         | 20 (54.1)        | 17 (45.9)        |         |
| 3                         | 7 (63.6)         | 4 (36.4)         |         |
| 4                         | 6 (75)           | 2 (25)           |         |
| 5                         | 0 (0)            | 1 (100)          |         |
| 6                         | 0 (0)            | 1 (100)          |         |
| 7                         | 1 (100)          | 0 (0)            |         |
| 8                         | 0 (0)            | 0 (0)            |         |
| 9                         | 0 (0)            | 0 (0)            |         |
| 10                        | 0 (0)            | 0 (0)            |         |

Table 2. Comparison of clinicopathologic characteristics of the patient population according to the needle size in ultrasonography-guided fine-needle aspiration. Note.—Data are number of items, with percentage in parentheses.
The following are the limitations of our study. First, there was a small sample size. Furthermore, the mean nodule size was different between two groups. This may be associated with a sampling bias. Second, 21 FNA cases with purely calcified thyroid nodules, thyroid cysts, or partially cystic thyroid nodules were excluded. Third, we did not investigate all the gauges of needles available (21 to 27-gauge). Fourth, the length of the 25-gauge needle was longer than that of the 23-gauge needle. This difference may have resulted in a bias. Finally, the histopathologic results of the 99 STNs were not investigated, as most of them were not confirmed by surgery.

In conclusion, the study results demonstrated that no significant differences were found in the cytological adequacy, cellularity, and pain scores between 23- and 25-gauge needles in US-guided FNA of STNs using LBC. Thus, we believe that both 23- and 25-gauge needles can be used in US-guided FNA of STNs using LBC.

Data Availability
The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

References
1. Fadda, G. & Rossi, E. D. Liquid-based cytology in fine-needle aspiration biopsies of the thyroid gland. Acta Cytol. 5, 389–400 (2011).
2. Geers, C. & Bourgain, C. Liquid-based FNAC of the thyroid: a 4-year survey with Surepath. Cancer (Cancer Cytopathol). 119, 58–67 (2011).
3. Chong, Y., Ji, S. J., Kang, C. S. & Lee, E. J. Can liquid-based preparation substitute for conventional smear in thyroid fine-needle aspiration? A systematic review based on meta-analysis. Endocr Connect. 6, 817–829 (2017).
4. Mygdakos, N., Nikolaidou, S., Tsivitaki, A. & Tamiolakis, D. Liquid Based Preparation (LBP) cytology versus Conventional Cytology (CS) in FNA samples from breast, thyroid, salivary glands and soft tissues. Our experience in Crete (Greece). Rom J Morphol Embryol. 50, 245–250 (2009).
5. Nagarajan, N., Schneider, E. B., Ali, S. Z., Zeiger, M. A. & Olson, M. T. How do liquid-based preparations of thyroid fine-needle aspiration compare with conventional smears? An analysis of 5475 specimens. Thyroid. 25, 308–313 (2015).
6. Tripathy, K., Misra, A. & Ghosh, J. K. Efficacy of liquid-based cytology versus conventional smears in FNA samples. J Cytol. 32, 17–20 (2015).
7. Abraham, T. M., Morenas, A. D. L., Lee, S. L. & Safer, J. D. In thyroid fine-needle aspiration, use of bedside-prepared slides significantly increased diagnostic adequacy and specimen cellularity relative to solution-based samples. Thyroid. 21, 237–242 (2011).
8. de Koster, E. J. et al. Thyroid ultrasound-guided fine-needle aspiration: the positive influence of on-site adequacy assessment and number of needle passes on diagnostic cytology rate. Acta Cytol. 60, 39–45 (2016).
9. Degirmenci, B. 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. 62, 798–803 (2007).
10. Cappelli, C. et al. Complications after fine-needle aspiration cytology: a retrospective study of 7449 consecutive thyroid nodules. Br J Oral Maxillofacial Surg. 55, 266–269 (2017).
11. Jung, S. J., Kim, D. W. & Back, H. J. Comparison Study of the Adequacy and Pain Scale of Ultrasound-Guided Fine-Needle Aspiration of Solid Thyroid Nodules with a 21- or 23-Gauge Needle for Liquid-Based Cytology: a Single-Center Study. Endo pathol. 29, 30–34 (2018).
12. Kim, D. W. et al. Ultrasonography-guided fine-needle aspiration cytology for thyroid nodules: an emphasis on one-sampling and biopsy techniques. Diagn Cytopathol. 40, E48–54 (2012).
13. Baloch, Z. W. 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. 36, 425–437 (2008).
14. Vivero, M., Renshaw, A. A. & Krane, J. F. Adequacy criteria for thyroid FNA evaluated by ThinPrep slides only. Cancer. 125, 534–543 (2017).
15. Kim, M. J. et al. US-guided fine-needle aspiration of thyroid nodules: indications, techniques, results. Radiographics. 28, 1869–1886 (2008).
16. Tangpricha, V. et al. 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. 11, 973–976 (2001).

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Author Contributions
D.W.K. made study concept and design, and Y.J.L. wrote the manuscript draft. All authors took part in the previous journal research and investigating clinical (Y.J.L. and D.W.K.), radiologic (Y.J.L., G.W.H., Y.J.H., J.W.B., H.J.C. and Y.J.C.), or pathologic (S.J.J.) data. H.J.B. performed statistical analysis. All authors read and approved the final manuscript.

Additional Information
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