Comparison of cytological adequacy in ultrasound-guided fine-needle aspiration of thyroid nodules with different numbers of needle passes

Q.-Y. CHEN¹, T. ZHANG¹, W.-G. LI², M.-L. LIANG¹, C.-Z. LI¹, D.-D. SONG¹, Z.-F. LI¹, D.-Y. PAN¹, S.-D. HU¹, Y.-Q. SONG¹, Z. CHEN¹

¹Department of Endocrinology, the Third Affiliated Hospital of Southern Medical University, Guangzhou, China
²Department of Endocrinology, the Second Affiliated Hospital, Guangzhou Medical University, Guangzhou, China

Abstract. – OBJECTIVE: The aim of this study was to compare the cytological adequacy rates of different needle passes in ultrasound-guided fine-needle aspiration biopsy of thyroid nodules and, thus, to help establish the criterion for selecting the number of needle passes according to the characteristics of thyroid nodules.

PATIENTS AND METHODS: This single-center and randomized prospective study involved 207 consecutive patients with 240 solid or predominantly solid thyroid nodules. These nodules were randomly divided into a 1-pass group, a 2-pass group, and a 3-pass group. Then the nodules were sent for cytopathological diagnosis, and cytological results were classified according to the Bethesda classification. Bethesda I was defined as inadequate, and Bethesda II-IV were defined as adequate. Then the cytological adequacy rates of different groups were compared.

RESULTS: In total, 221 nodule specimens were considered as adequate and 19 nodule specimens inadequate. The overall adequacy rate was 92.1%. However, there were no significant differences among the 1, 2, and 3-pass groups in terms of adequacy rates (91.3%, 92.5%, and 92.5%, respectively).

CONCLUSIONS: The number of needle passes does not significantly affect the cytological adequacy in ultrasound-guided fine-needle aspiration biopsy of solid or predominantly solid thyroid nodules. The cytological adequacy of one-needle pass is comparable to those of two and three-needle passes.

Key Words: Fine-needle aspiration of thyroid nodules, Number of needle passes, Cytological adequacy rate.

Introduction

Thyroid cancer is one of the five major types of tumors among women¹. Thyroid nodules, another common disease, can be detected by ultrasonic imaging in up to 76% of people². Thyroid nodules are mostly benign, but about 5-15% are malignant. The diagnosis and treatment of thyroid nodules are influenced by many factors, especially the result of ultrasound-guided fine-needle aspiration cytological diagnosis³. Ultrasound-guided fine-needle aspiration biopsy (FNAB), which shows high sensitivity and specificity⁴, is a reliable and minimally invasive diagnostic method to differentiate malignant and benign thyroid nodules⁵.

However, its application is limited by the high nondiagnostic rate. Reportedly, 2-34% of thyroid nodules cannot be definitely and pathologically diagnosed⁶. About 2-14% of the thyroid nodules without a definite diagnosis are considered malignancies in the surgically resected tissues⁷,⁸. The nondiagnostic result may lead to delayed treatment, ineffective diagnostic thyroidectomy, or repeated fine-needle aspiration. And then repeated fine-needle aspiration may result in anxiety and a high economic burden on patients.

Retrospective studies⁹-¹¹ have demonstrated that the cytological adequacy rate is affected by multiple factors, including the characteristics of nodules, the operator’s experience, needle gauge, way of aspiration, the conduct of rapid on-site assessment, and the conduct of anesthesia. Some prospective randomized controlled trials¹²-¹⁵ have identified the effects of needle gauges, rapid on-site assessment, and aspiration skills on the
cytological adequacy rate. However, the specific number of needle passes of FNAB for high cytological adequacy remains in doubt. The number of needle passes required for US-guided FNA of thyroid nodules also differs among retrospective studies\(^6,17\). Hence, we performed a randomized and controlled prospective study to further explore the effect of needle pass number on the cytological adequacy rate. This study will better guide clinical works.

**Patients and Methods**

**Patients**

This prospective study was approved by the Chinese Clinical Trial Registry Ethics Committee (ChiERCT20210374). All experiments were performed in accordance with relevant guidelines. Informed written consent was obtained from all patients prior to the US-guided FNA. From September 2021 to March 2022, the same endocrinologist with ten years of experience in US-guided FNA of thyroid nodules performed US-guided FNA in 232 consecutive patients (168 females, 64 males; mean age: 46.9±13.7 years, ranging from 18 to 76 years). All patients met the conditions of fine-needle aspiration recommended in the Guidelines for the Diagnosis and Management of Thyroid Nodules issued by American Association of Clinical Endocrinologists (AACE)\(^18\). Firstly, 270 thyroid nodules that were ≥5 mm in the largest diameter were diagnosed (mean size: 2.12±1.28 cm, ranging from 0.5 to 6.5 cm). Among them, predominantly cystic thyroid nodules (n=30) were excluded. Ultimately, 240 solid or predominantly solid thyroid nodules (mean size: 2.01±1.22 cm, ranging from 0.5 to 5.7 cm) in 207 patients (150 women and 57 men; mean age: 46.4±13.4 years, ranging from 19 to 76 years) were included. Predominantly solid thyroid nodule was defined as thyroid nodule with a solid component accounting for ≥50% of the total volume.

**US-Guided FNA Procedure**

All biopsies were performed by an endocrinologist with extensive experience in US-guided FNA of thyroid nodules. The number of needle passes (1, 2, 3 passes) was randomly assigned (random digits were used in grouping, and the random digit seed was 20200811). Before FNAB, the patient was maintained in the supine position with an extended neck to fully expose the thyroid gland, and the skin was sterilized with a povidone-iodine solution. Under a Logiq P6 ultrasound guidance instrument (GE Corp., Seongnam, Gyeonggi, Korea), a PA23/08 23G biopsy needle (Gallini Corp., Mirandola, Modena, Italy) was accurately inserted into the suspicious part of the thyroid nodule (e.g., solid, microcalcified, hypoechoic area) with no vacuum aspiration, and then moved back and forth for 20 times for specimen collection by the endocrinologist. The collected material was directly smeared on a glass slide immediately and fixed in 95% ethyl alcohol. Then the specimens were sent to the Department of Pathology for cytopathological diagnosis.

**Cytological Analysis**

The cytopathologic diagnosis was classified according to the Bethesda classification of thyroid cytopathology\(^19\) as follows: (I) nondiagnostic, (II) benign, (III) atypia of undetermined significance, (IV) suspicious for a follicular neoplasm, (V) suspicious for malignancy, or (VI) malignant. Here, Bethesda I, and Bethesda II-VI were considered inadequate and adequate, respectively.

**Statistical Analysis**

Data were analyzed with SPSS 26.0 (IBM Corp., Armonk, NY, USA). The data in normal distribution were tested using the Kolmogorov-Smirnov test. The quantitative data that obeyed normal distribution were presented as mean, and otherwise as median and interquartile range. Qualitative data were presented as percentages. The cytological adequacy rates of different groups were compared by the \(\chi^2\) test, and those for small cell values were examined using Fisher’s exact test. The patients’ ages were compared using non-parametric tests (Kruskal-Wallis’ test, H test). All the tests were 2-tailed, and \(p<0.05\) was considered statistically significant.

**Results**

All patients completed the US-guided FNA of thyroid nodules. There was no special discomfort during or after the procedure, except for mild pain. One-, 2- or 3-pass US-guided FNA each was performed for 80 (33.3%) thyroid nodules. The demographic characteristics of the three groups and the characteristics of nodules under ultrasound are presented in Table I. No significant between-group differences were found in age, gender, nodule diameter, components, echoes, shape, edges, calcification, or blood supply, indicating these data are comparable among groups.
Of the 240 thyroid nodules, 221 showed adequate cytology, and 19 had inadequate cytology, showing an overall cytological adequacy rate of 92.1%.

The cytological adequacy rates in the 1-, 2- and 3-pass groups were 91.3% (73/80), 92.5% (74/80), and 92.5% (74/80) respectively, with no significant differences among groups ($p=1.000$) (Figure 1). The thyroid nodules were divided by the maximum diameter ($D_{\text{max}}$) into group A (0.5 cm ≤ $D_{\text{max}}$ < 1.0 cm), group B (1.0 cm ≤ $D_{\text{max}}$ < 2.0 cm), group C (2.0 cm ≤ $D_{\text{max}}$ < 3.0 cm), and group D ($D_{\text{max}}$ ≥ 3.0 cm). In group A, the cytological adequacy rates of 1, 2 and 3 passes were 81.3% (13/16), 94.4% (17/18), and 81.3% (13/16), respectively. And the cytological adequacy rates were 96.0% (24/25), 90.6% (29/32), and 93.3% (28/30), respectively, in group B, 95.5% (21/22), 91.7% (11/12), and 100% (17/17) respectively in group C, 88.2% (15/17), 94.4% (17/18), and 94.4% (17/18) respectively in group D. No significant difference was found among different numbers of needle passes.

### Table I. Comparison of demographic characteristics of the patient population and characteristics of nodules under ultrasound in three groups.

<table>
<thead>
<tr>
<th>Index</th>
<th>1-pass No. (%) (n = 80)</th>
<th>2-pass No. (%) (n = 80)</th>
<th>3-pass No. (%) (n = 80)</th>
<th>$p$-value</th>
</tr>
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<tbody>
<tr>
<td>Age/years</td>
<td>46.3 ± 14.0</td>
<td>46.6 ± 13.9</td>
<td>46.3 ± 12.4</td>
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<tr>
<td>Gender</td>
<td></td>
<td></td>
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<td>0.062</td>
</tr>
<tr>
<td>Female</td>
<td>66 (82.5)</td>
<td>53 (66.2)</td>
<td>58 (72.5)</td>
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</tr>
<tr>
<td>Male</td>
<td>14 (17.5)</td>
<td>27 (33.8)</td>
<td>22 (27.5)</td>
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<tr>
<td>Nodule diameter/cm</td>
<td></td>
<td></td>
<td></td>
<td>0.667</td>
</tr>
<tr>
<td>0.5 ≤ $D_{\text{max}}$ &lt; 1.0</td>
<td>16 (20.0)</td>
<td>18 (22.5)</td>
<td>16 (20.0)</td>
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<tr>
<td>1.0 ≤ $D_{\text{max}}$ &lt; 2.0</td>
<td>25 (31.3)</td>
<td>32 (40.0)</td>
<td>30 (37.5)</td>
<td></td>
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<tr>
<td>2.0 ≤ $D_{\text{max}}$ &lt; 3.0</td>
<td>22 (27.4)</td>
<td>12 (15.0)</td>
<td>16 (20.0)</td>
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</tr>
<tr>
<td>$D_{\text{max}}$ ≥ 3.0</td>
<td>17 (21.3)</td>
<td>18 (22.5)</td>
<td>18 (22.5)</td>
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</tr>
<tr>
<td>Components</td>
<td></td>
<td></td>
<td></td>
<td>0.280</td>
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<tr>
<td>Solid</td>
<td>49 (61.3)</td>
<td>50 (62.5)</td>
<td>58 (72.5)</td>
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<tr>
<td>Predominantly solid</td>
<td>31 (38.7)</td>
<td>30 (37.5)</td>
<td>22 (27.5)</td>
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<tr>
<td>Echo</td>
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<td>0.436</td>
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<tr>
<td>Hypoechoic</td>
<td>42 (52.5)</td>
<td>36 (45.0)</td>
<td>46 (57.4)</td>
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<tr>
<td>Isoechoic</td>
<td>35 (43.8)</td>
<td>40 (50.0)</td>
<td>33 (41.3)</td>
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<tr>
<td>Hyperchoic</td>
<td>3 (3.7)</td>
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<td>&lt; 1</td>
<td>73 (91.3)</td>
<td>70 (87.5)</td>
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<td>≥ 1</td>
<td>7 (8.7)</td>
<td>10 (12.5)</td>
<td>11 (13.7)</td>
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<td>Micro</td>
<td>19 (23.8)</td>
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<td>Macro</td>
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<tr>
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<td>Edges</td>
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<td>38 (47.5)</td>
<td>45 (56.3)</td>
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<tr>
<td>Unclear</td>
<td>30 (37.5)</td>
<td>42 (52.5)</td>
<td>35 (43.7)</td>
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</tbody>
</table>

$D_{\text{max}}$ is maximum diameter.

**Figure 1.** Adequacy of samples obtained with different numbers of needle passes.
Cytological adequacy in ultrasound-guided fine-needle aspiration of thyroid nodules

of passes within the same group ($p=0.412, 0.872, 0.713, 0.680$ respectively) (Figure 2).

The thyroid nodules were also separated by the components into a solid group and a predominantly solid group. The cytological adequacy rates after treatment with 1, 2 and 3 passes were $93.5\%$ (29/32), $93.3\%$ (28/30), and $100.0\%$ (22/22), respectively, in the predominantly solid group, $89.8\%$ (44/49), $92.0\%$ (46/50), and $89.7\%$ (52/58) respectively in the solid group. No significant difference was found within the same group ($p=0.555, 0.890$ respectively) (Figure 3).

**Discussion**

US-guided FNA cytopathological diagnosis of thyroid nodules is a safe, effective, and minimally invasive method. It is highly sensitive and specific in assessing benign and malignant thyroid nodules. However, there is no clear consensus regarding the specific number of needle passes required to ensure the adequacy of samples for this method. Moreover, no prospective randomized controlled data are available about the effects of needle pass number on the cytological adequacy rate.

A retrospective study shows that in cases where rapid on-site adequacy assessment cannot be performed, a minimum of three needle passes is recommended to ensure the adequacy of FNA. Some scholars suggest a minimum of two and a maximum of three needle passes for FNA adequacy with the right technique and preparation. In some cases, up to 5 passes were applied, because the increase of discomfort surpassed the slight increment of sampling satisfaction. The
addition of one more pass intensifies pains and discomfort and raises medical expenses, and may even increase the risks of infection, bleeding, and other relevant complications.

In our study, the cytological adequacy rate was 92.1%, which is close to the result of 92.05% reported by Ullah et al. Moreover, the cytological adequacy rates are not significantly different among one, two, and three-needle passes, indicating that one needle pass can achieve the cytological adequacy rate of two and three-needle passes. Thus, we preliminarily hold that for solid or predominantly solid thyroid nodules, the cytological adequacy rate is more affected by the operator’s experience compared to the number of needle passes.

There is no consensus on whether FNA is required for nodules in diameter below 1.0 cm. The American Thyroid Association Management Guidelines suggest that nodules in diameter smaller than 1.0 cm are difficult to locate and diagnose and cannot be treated by FNA even if malignant tumors are suspected. The American Association of Clinical Endocrinologists recommends US-guided FNA for nodules in size of 0.5 to 1 cm with high-risk factors. In China, aspiration biopsy is also commonly applied to nodules in size below 1.0 cm. Reportedly, the cytological inadequacy rate is high for nodules in diameter <1.0 cm or larger size (≥3.0 cm). In the study, we included 50 nodules in diameter <1.0 cm, and the overall cytological adequacy rate was 92.1%. The possible reason for such a high rate is that the experienced operator in our hospital can successfully obtain components, rather than peripheral thyroid tissues, from nodules in diameter <1.0 cm.

Moreover, subgroup analyses by diameters and by components show that the number of needle passes is not related to the cytological inadequacy rate. This conclusion shall be further investigated using larger-size experiments to clarify the properties of thyroid nodules and to work out a standard for selecting the number of needle passes, which will better guide clinical works.

Limitations
Our study has some limitations. This is a single-center randomized and controlled study, which shall be validated by involving more centers.

In addition, a lack of histopathological diagnosis may bring some false positives and false negatives for the diagnosis of ultrasound-guided fine-needle aspiration biopsy.

Conclusions
For solid or predominantly solid thyroid nodules, the number of needle passes does not significantly affect the cytological adequacy in ultrasound-guided fine-needle aspiration. The cytological adequacy rate of one needle pass is comparable to those of two and three-needle passes.

Conflict of Interest
The Authors declare that they have no conflict of interests.

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Authors’ Contribution
Q.-Y. Chen: Conceptualization; methodology; investigation; formal analysis; writing-original draft. T. Zhang: Conceptualization; writing-review, and editing. W.-G. Li: Data curation; Writing-review, and editing. M.-L. Liang: Data curation; software. C.-Z. Li: Formal analysis. D.-D. Song: Project administration. Z.-F. Li: Visualization. D.-Y. Pan: Resources. S.-D. Hu: Investigation. Y.-Q. Song: validation. Z.Chen: Conceptualization; Resource; supervision; writing-review, and editing.

Ethics Approval
This study was approved by the Chinese Clinical Trial Registry Ethics Committee (ChiERCT20210374). The clinical trial was registered on the Chinese Clinical Trial Registry (https://www.chictr.org.cn/). The clinical trial number was ChiCTR2100049103.

Informed Consent
Informed written consent was obtained from all patients prior to the US-guided FNA.

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