

## Examination of Selective Low-pressure Fine Needle Aspiration Cytology Under Ultrasound Guidance

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### ABSTRACT

Cytology by fine-needle cytology is indispensable for diagnosing head and neck tumor, especially for thyroid nodule. There are two methods of fine needle cytology; one of fine-needle aspiration cytology (FNAC) and another of fine-needle non-aspiration cytology (FNNAC). These previous procedures has each disadvantage such as the mixing of blood or low yield of cells. We proposed a new technique: selective low-pressure fine needle aspiration cytology (SLOP-FNAC) to overcome the backwards of previous procedures. We used the scoring system by Mair et al. to evaluate smear quality of specimens obtained with FNNAC and SLOP-FNAC. SLOP-FNAC smears exhibited higher scores in amount of cellular material, degree of cellular degeneration and cell yield, and retention of appropriate architecture compared to FNNAC smears. The SLOP-FNAC smears scored significantly higher for amount of cellular material and retention of appropriate architecture evaluated ( $P = 0.0261$  and  $P = 0.0024$ , Student's *t*-test). SLOP-FNAC may be a useful cell sampling technique that reduces blood contamination while securing a high cell yield with maintaining tissue structure.

**Key words** cytology; fine needle aspiration cytology; fine needle non-aspiration cytology; ultrasonography; thyroid tumor

Fine-needle cytology is indispensable for diagnosing head and neck tumor, especially for thyroid nodule. There are two methods of fine needle cytology: applying

negative pressure and not applying negative pressure. The former is called fine-needle aspiration cytology (FNAC) and the latter is called fine-needle non-aspiration cytology (FNNAC). FNAC, the conventional procedure done by applying negative pressure, has the disadvantage of blood contamination. Briffod et al. developed FNNAC to improve this sample defect rate.<sup>1</sup> FNNAC, the procedure without aspiration, prevents the mixing of blood with other substances; however, sometimes insufficient sampling of diagnostic cells occurs.<sup>2</sup> Therefore, we proposed a new technique called selective low-pressure fine needle aspiration cytology (SLOP-FNAC) to mitigate problems associated with FNAC and FNNAC. This procedure holds the possibility of reducing blood contamination while securing sufficient cell collection by aspiration biopsy with stable low negative pressure. We compared our new method to FNNAC by using specimen smear analysis with the scoring system developed by Mair et al.<sup>3</sup>

### MATERIALS AND METHODS

#### The procedure of SLOP-FNAC

The mechanism of SLOP-FNAC is enabled when stable low negative pressure is supplied by connecting a suction machine and the examiner can switch from non-aspiration to aspiration easily using a three-way stopcock. The stable negative pressure requires only one forward-backward movement of the needle tip, while conventional FNAC and FNNAC require several forward-backward movements of the needle tip. A three-way stopcock is attached to a 21-gauge needle and connected to an aspirator equipped in the treatment room. The mechanism of paracentesis is performed while keeping the side tube closed and maintaining suction at low pressure. The puncturing procedure is as follows (Fig. 1A):

- i) The side port is kept open without suction until the needle tip is positioned at the site of the tumor;
- ii) When the needle tip reaches the tumor, the side tube

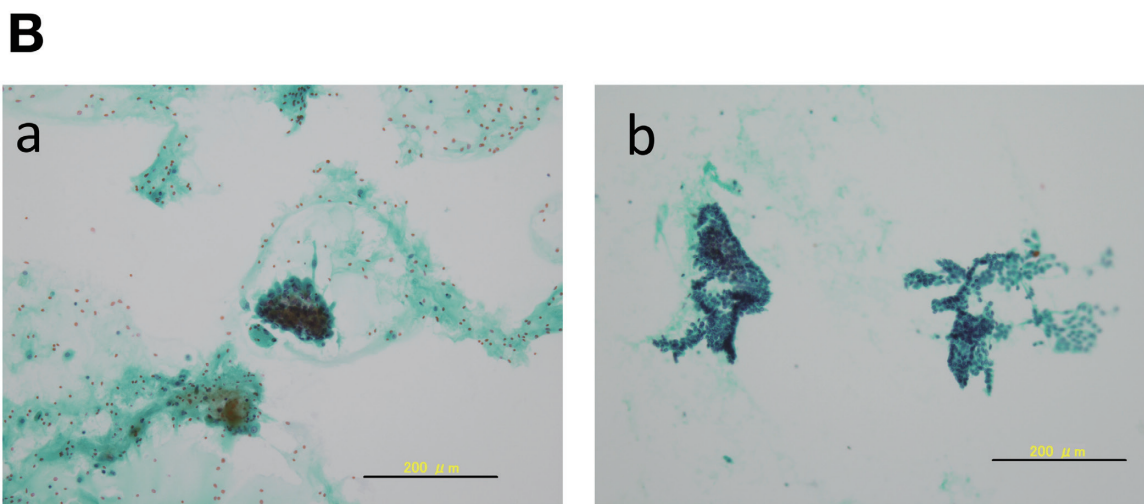
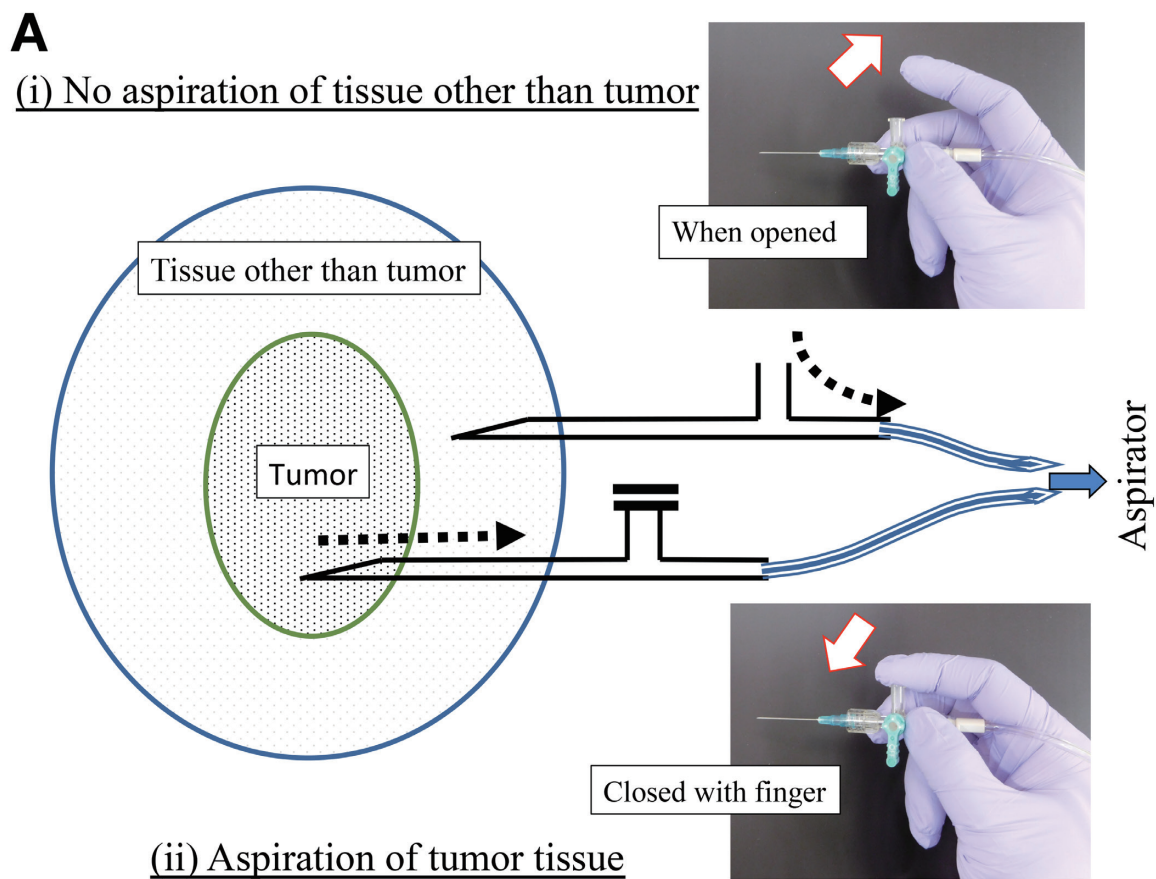
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Abbreviations: FNAC, fine needle aspiration cytology; FNNAC, fine needle non-aspiration cytology; SLOP-FNAC, selective low-pressure fine needle aspiration cytology



**Fig. 1. A:** Selective Low-pressure FNAC “SLOP-FNAC. **(i)** The side port remains open without suction until the needle tip is positioned at the site of the tumor. **(ii)** When the needle tip reaches the tumor, the side port is closed with the index finger and tumor tissue is aspirated. **B:** Cytological appearances of thyroid papillary carcinoma. **(a)** FNNAC. Amount of cellular material is sufficient for diagnosis and retention of appropriate architecture is some preservation. Papanicolaou stain, bar = 200  $\mu\text{m}$ . **(b)** SLOP-FNAC. Amount of cellular material is abundant and retention of appropriate architecture is excellent. Papanicolaou stain, bar = 200  $\mu\text{m}$ . FNAC, fine needle aspiration cytology; SLOP-FNAC, selective low-pressure fine needle aspiration cytology, FNNAC, fine needle non-aspiration cytology.

- is closed with the index finger;
- iii) The needle is drilled forward and backward once while twisting;
- iv) The index finger is released, to open the side tube, and the needle tip is removed.

In this manner, low-pressure aspiration can be performed at a specific location to allow for fine manipulation.

**Setting negative pressure**

Strong aspiration of a tumor with rich blood flow, like thyroid tumors, can cause blood contamination. Blood contamination makes cytological decision making difficult. Therefore, we set up the constant low pressure applied in our procedure to prevent blood contamination according to the following: We measured the suction pressure of a regular syringe of 20 mL. The suction pressure was approximately 5 kPa when the plunger was pulled very slowly, and approximately 20 kPa when the plunger was pulled quickly. Therefore, we set the suction pressure to 10 –15 kPa which is comparable with the pressure obtained through constant, gradual suction with a syringe.

**Clinical experiment of our procedure**

All the procedures were performed by the same endocrine surgeon in our outpatient hospital department. Ultrasonography was used during the procedure. Slides were alcohol-fixed and sent to our laboratory for staining and evaluation. All the slides were stained with Papanicolaou stain.

This study was approved by the Institutional Re-

view Board of Kanazawa Medical University (Approval Number I050) and was performed in accordance with the Declaration of Helsinki.

**Patients**

Three patients underwent FNNAC, and five underwent SLOP-FNAC. The smear quality of the specimens from these patients was evaluated. In this trial, we targeted solid tumors of 5 mm or more in the thyroid.

**Evaluation method of smear quality in FNNAC and SLOP-FNAC**

The scoring system of Mair et al. was used to evaluate the smear quality of cytological specimens<sup>3</sup> (Table 1). Two pathologists and two technicians performed the evaluation. The evaluation criteria in the scoring system of Mair et al. are background blood/clot, amount of cellular material, degree of cellular degeneration, degree of cellular trauma, and retention of appropriate architecture. Higher scores are better. We calculated individual and total scores for the smears. The mean score for each evaluation method was statistically examined using the Student *t*-test, and a significant difference was set at *P* < 0.05. All statistical analyses were conducted using IBM SPSS version 21.0.

**RESULTS**

**Evaluation method of smear quality in FNNAC and SLOP-FNAC**

We used the method of Mair et al. for evaluation of smear quality. All the evaluators assigned higher scores to the SLOP-FNAC smears than to the FNNAC smears.

**Table 1. The Mair S et al. scoring system and individual evaluation results**

Criterion	Qualitative description	Score	FNNAC (n = 3) Mean (SD)	SLOP-FNAC (n = 5) Mean (SD)	P-value
Background blood / clot	Large amount / great compromise to diagnosis	0			0.3388
	Moderate / diagnosis possible	1	1.083 ± 0.289	1.200 ± 0.410	
	Minimal / diagnosis easy; specimen of textbook quality	2			
Amount of cellular material	Minimal to absent / diagnosis not possible	0			0.0261*
	Sufficient for diagnosis	1	0.833 ± 0.389	1.350 ± 0.489	
	Abundant / diagnosis simple	2			
Degree of cellular degeneration	Marked / diagnosis impossible	0			0.0538
	Moderate / diagnosis possible	1	1.083 ± 0.289	1.450 ± 0.510	
	Minimal / good preservation; diagnosis easy	2			
Degree of cellular trauma	Marked / diagnosis impossible	0			0.6742
	Moderate / diagnosis possible	1	1.583 ± 0.515	1.450 ± 0.510	
	Minimal / diagnosis easy	2			
Retention of appropriate architecture	Minimal to absent / non diagnostic	0			0.0024**
	Moderate / some preservation	1	0.667 ± 0.494	1.250 ± 0.444	
	Excellent architecture display, closely reflecting histology	2			

\**P* < 0.05, \*\**P* < 0.01

FNNAC, fine needle non-aspiration cytology; SLOP-FNAC, selective low-pressure fine needle aspiration cytology.

According to the mean score for each smear, the SLOP-FNAC smears exhibited higher scores for amount of cellular material, degree of cellular degeneration and cell yield, and retention of appropriate architecture than the FNNAC smears (Fig. 1B). Notably, the SLOP-FNAC smears scored significantly higher for amount of cellular material and retention of appropriate architecture ( $P = 0.0261$  and  $P = 0.0024$ , respectively; Table 1).

## DISCUSSION

FNNAC was invented to overcome the drawback of blood contamination, but it became clear that FNNAC has another drawback: low yield of cells. Hongming et al. performed a meta-analysis of FNAC and FNNAC, and concluded that the two techniques showed no significant differences regarding specimen deficiency, smear quality, or diagnostic accuracy and that both techniques are necessary for evaluating thyroid gland nodules. By combining these techniques, higher quality smears can be obtained.<sup>4</sup> Thus, we proposed a new technique called SLOP-FNAC that combines the good points of the previous two procedures and strategies to overcome simultaneously their disadvantages, such as the mixing of blood and low yield of cells.

Amit et al. used a scoring system proposed by Mair et al.<sup>5</sup> to evaluate smears of specimens obtained using FNAC and FNNAC.<sup>5</sup> In this study, we used the scoring system of Mair et al. to assess smears of specimens obtained using FNNAC and SLOP-FNAC. The evaluators assigned high scores for SLOP-FNAC in most of the evaluated items. SLOP-FNAC scored high in yield of cells and tissue structure, which were problematic in the FNNAC smears. Additionally, the score of background blood/clot of SLOP-FNAC was not inferior to that of FNNAC although the SLOP-FNAC procedure applied negative pressure. This means the SLOP-FNAC procedure may be able to prevent blood contamination.

Our results show that SLOP-FNAC can perform suction without damaging cells owing to its low pressure, prevent blood contamination, and produce a high yield of cells necessary for diagnosis. The cell structure of the specimen collected using the SLOP-FNAC method was maintained, making it easy to determine the architecture of the tissue.

To reduce the sample defect rate, some authors explained that they check the samples on the slide glass at

the precise moment and repeat testing to obtain a sufficient yield of cells for diagnosis.<sup>6</sup> However, we think that puncturing the tumor membrane results in barrier disruption, which can lead to the invasion and seeding of tumor cells into surrounding tissues. Additionally, the repeated puncturing increases a patient's burden. Therefore, the significance of the present technique, which collects numerous cells in one pass is recognized for its importance.

In this study, some limitations should be acknowledged. One problem is that bias was generated because only one examiner performed the examination. The purpose was to avoid the inconsistency by multiple examiners in our first study of SLOP-FNAC. Another problem is that the number of participants was small. Further studies are needed to verify the superiority of our procedure.

In summary, SLOP-FNAC may be a useful cell sampling technique that reduces blood contamination while securing a high cell yield with maintaining tissue structure.

*The authors declare no conflict of interest.*

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