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Comparing cytological adequacy between conventional smear and liquid-based cytology in ultrasound-guided fine needle aspiration of thyroid nodules: A prospective study

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Original article

Comparing cytological adequacy between conventional smear and liquid-based cytology in ultrasound-guided fine needle aspiration of thyroid nodules: A prospective study

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Background: The major problem of conventional smear for ultrasound-guided fine needle aspiration (FNA) of thyroid nodules is the sample inadequacy. Liquid-based cytology (LBC) is claimed to perform better than the conventional smear (CS) in reducing sample inadequacy. However, the evidence of better adequacy in LBC is unclear.

Objective: This study is the first prospective study aiming to compare sample adequacy in CS and LBC using ThinPrep system with adequacy criteria based on the 2017 Bethesda System for Reporting Thyroid Cytopathology.

Methods: One hundred and twenty thyroid nodules were prospectively recruited for ultrasound-guided FNA from March to September 2019. The sample from each nodule was prepared in both CS and LBC using a randomised needle size between 25 or 23G. The cytological adequacy for each method was reviewed by only one pathologist.

Results: There was no significant difference in adequacy rate between the CS and LBC group (57.5% vs. 53.3%, $P = 0.371$). Univariate and multivariate analysis showed only two factors to be significantly associated with adequacy, including the presence of internal vascularity on ultrasound (adjusted RR = 1.3, $P = 0.038$) and the use of 25G needles compared with 23G needles (adjusted RR = 1.4, $P = 0.013$).

Conclusion: This prospective study did not demonstrate the superiority of LBC over CS. The presence of internal vascularity on ultrasound is a potential predictor of FNA adequacy. The use of a 25G needle may be recommended rather than a 23G needle in thyroid FNA.

Keywords: FNA, thyroid nodule, liquid based cytology, adequacy.

Ultrasound-guided fine needle aspiration (FNA) of thyroid nodules is a commonly used investigation to detect thyroid malignancy. Conventional smear (CS) for cytology is the main method for evaluation of the FNA sample. The major problem of conventional smear is the large number of non-diagnostic FNA, which was reported to range from 63.0 – 98.0%.[1, 2] Meanwhile, the Bethesda System for Reporting Thyroid Cytopathology recommends an ideal adequacy rate of more than 90.0% for all thyroid FNA.[3]

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Liquid-based cytology (LBC) is claimed to perform better than conventional smear in reducing the inadequacy of thyroid FNA by removing the obscuring red blood cells and cell debris from needle trauma.[4, 5] Currently, there are two widely used systems of LBC: ThinPrep and SurePath. However, evidence of the superiority of the two systems of LBC is not well established. A meta-analysis by Chong Y, *et al*. showed a higher adequacy rate of ThinPrep compared with CS in FNA of thyroid nodules (76.0% vs. 66.6%, $P < 0.01$). [1] Unfortunately, the majority of the included studies (5 of the 7 studies) were retrospective. There was only one prospective study supporting the use of ThinPrep, by Cavaliere A, *et al.* The study was limited by the abnormally high percentage of benign cytological diagnoses because it was conducted in an endemic goitre area.[6] Moreover, the adequacy criteria in the study by Cavaliere A, *et al.* was based on those of the British

Thyroid Association 2002, which did not allow some exceptional findings to be adequate, and were in contrast to the current Bethesda criteria.[7, 8] As for SurePath, although the meta-analysis by Chong Y, *et al.* showed a higher rate of adequacy than CS, this result was from limited data (only two comparative studies).[1] In addition, there were opposite results from a more recent prospective study using the SurePath system by Liu *et al*., showing a significantly higher adequacy rate in CS (63.8%) than in LBC (57.2%).[9] Therefore, there is a need to investigate the real advantage of LBC in reducing the inadequacy of thyroid FNA.

This study is the first prospective study aiming to compare sample adequacy between CS and LBC in ultrasound guided FNA of thyroid nodules using ThinPrep with adequacy criteria based on the 2017 *Bethesda System for Reporting Thyroid Cytopathology.*

Materials and methods

The institutional review board (IRB) of the Faculty of Medicine, Chulalongkorn University has approved this prospective study. We recruited all patients who were sent for ultrasound-guided FNA of thyroid nodules aged more than 18 at the Interventional Radiology Unit, Department of Radiology, King Chulalongkorn Memorial Hospital from March 2019 to September 2019. The nodules at the surgical bed from prior total/subtotal thyroidectomy were excluded. There was no other selection criterion, so this study was a continuous unselected cohort. Informed consent was obtained from all subjects.

FNA procedure

All FNAs were performed under ultrasound guidance by rotating fellows and interventional radiologists. Under aseptic technique and the application of local anaesthesia, a high-resolution linear-array transducer with a sterile plastic cover was used for ultrasound examination. The location, size and characteristics of each nodule were documented by each operator.

The needle size was randomised between 23 or 25 G needle for each nodule. The needle was attached to a 10 mL syringe. A freehand technique was used under real-time ultrasound guidance. The technique could be either aspiration or non-aspiration (capillary action technique), or both, depending on the operator. The number of needle passes for each nodule was set to four. However, the number of needle passes could be adjusted by the operator to obtain a satisfactory amount and quality of specimen.

The specimen was divided into halves and prepared for CS and LBC. The sequence of sample preparation was also randomised, with either CS or LBC first. As for CS, the specimen was prepared in 4 slides and immersed in 95% alcohol, while it was rinsed in a tube containing 30 mL of methanol-based solution (Cytolyt) for LBC.

If more than one nodule in any one patient was indicated for FNA, the needle size (23 or 25 G) and sequence of sample preparation (CS first or LBC first) was randomised for each nodule separately.

Cytological analysis and interpretation

The LBC in this study was processed using ThinPrep2000™ system (Hologic Co., Marlborough, MA, USA). The system requires three main steps: 1) collection of the sample into Cytolyt™ solution; 2) after centrifugation, transferring the cell pellet into the second methanol-based solution (PreservCyt™); and, 3) putting the PreservCyt solution into the automated processor. The specimens would be filtered and transferred onto a slide. The final result is one slide for each lesion with all cells concentrated in the central area of the slide, which would be subsequently stained with the *Papanicolaou stain.* [10, 11]

The adequacy criteria were based on the 2017 Bethesda System for Reporting Thyroid Cytopathology.[8] As for a thyroid FNA specimen to be satisfactory for evaluation, at least six groups of benign follicular cells are required; each group was therefore composed of at least 10 cells. There are several exceptions to this requirement. Any specimen that contains abundant colloid is considered adequate, even if six groups of follicular cells are not identified. Whenever a specific diagnosis (e.g., lymphocytic thyroiditis [Hashimoto's thyroiditis]) can be made, and whenever there is any significant atypia, the specimen is considered adequate.

The cytological adequacy was reviewed by only one pathologist (Jitpasutham T.). The review of CS or LBC for each nodule was done in separate sessions and in randomized fashion. The pathologist was blinded to clinical and ultrasound information as well as the result of the other method. Intra-observer variability was tested by the second review of 20 randomly selected cases from each group by only one pathologist (Jitpasutham T.).

Outcomes

The primary outcome of this study was the adequacy of LBC and CS in ultrasound-guided FNA of thyroid nodules. The secondary outcome was to investigate the factors that affect the adequacy of thyroid FNA including nodule characteristics on ultrasound (solid/ predominantly solid / predominantly cystic, with/without internal vascularity, with or without micro / macro-calcifications and size) as well as the two needle sizes.

Statistical analysis

The assessment of demographic data of patients and nodule characteristics was performed using descriptive statistics. Data were expressed as mean ± standard deviation (SD). Adequacy rate comparison was analyzed using the McNemar Chi-square test for paired proportions. Subgroup analysis based on nodule characteristics and needle sizes was assessed using the McNemar exact test for paired proportions. Univariate and multivariate analysis using the Generalised Estimating Equation with binomial family and log-link was analyzed to determine the factor associated with the adequacy of FNA.

Intra-observer variability for cytological adequacy was calculated using the kappa test. All statistical analysis was performed by using STATA version 14.0 (StataCorp). A P – value < 0.05 was considered as significant difference.

Results

Demographic data

A total of 120 thyroid nodules from 89 patients were recruited, with a mean age of 54.0 ± 14.0 years old. The majority of nodules were from female patients (110 nodules, 91.7%).

Detail of FNA procedures

Nearly all of the procedures were performed by rotating fellows (98.0%). The mean number of needle passes was 2.8 times.

FNA result

The FNA result from both methods according to 2017 Bethesda System for Reporting Thyroid Cytopathology was presented in Table 1. In non-diagnostic cases, the cytological results were sub-grouped as cystic fluid content in 8 cases (17.4%), hypocellularity in 10 cases (21.7%) and bloody smear in 28 cases (60.9%).

Nodule characteristics

The mean size of nodules was 2.1 ± 1.4 cm with 60.0% being solid. The internal vascularity was found in 46.7% of nodules. In all, 27.5% of the nodules had micro-calcifications and 14.2% of the nodules had macro-calcifications (Table 2).

Adequacy rate comparison between CS and LBC

The adequacy in the CS group was higher than in the LBC group, but there was no statistical significance (57.5% vs. 53.3%, P = 0.371).

Subgroup analysis based on nodule characteristics

There was no significant difference in adequacy between CS and LBC in each group of nodule characteristics, including size, solidity, internal vascularity and calcification.

Subgroup analysis based on needle size

There was no significant difference of adequacy between CS and LBC in each needle size (23G and 25G).

Factors associated with adequacy of FNA

Univariate and multivariate analysis showed only two factors that show a significant association with adequacy including the presence of internal vascularity on ultrasound (adjusted RR = 1.3, P = 0.038) and the use of 25G needles compared with 23G needles (adjusted RR = 1.39, P = 0.013). The other factors were not significantly associated with adequacy (Table 3).

Table 1. FNA result from both methods according to the 2017 Bethesda System for Reporting Thyroid Cytopathology.

Diagnostic category		Number of nodules	Percentage
I	Non-diagnostic or unsatisfactory	46	38.3
II	Benign	46	38.3
III	Atypia of undetermined significance or follicular lesion of undetermined significance	17	14.2
IV	Follicular neoplasm or suspicious for a follicular neoplasm	5	4.2
V	Suspicious for malignancy	4	3.3
VI	Malignant	2	1.7

Table 2. Adequacy rate in CS and LBC with subgroup analysis based on nodule characteristics and needle sizes.

	Number of nodules (%)	Adequacy in CS (%)	Adequacy in LBC (%)	*P* - value
Overall adequacy	120	69 (57.5)	64 (53.3)	0.398
Nodule characteristic				
Size				
< 1 cm	24 (20.0)	12 (50.0)	11 (45.8)	1.000
1 - 2 cm	45 (37.5)	26 (57.8)	25 (55.6)	1.000
> 2 cm	51 (42.5)	31 (60.8)	28 (54.9)	0.549
Solidity				
Solid	72 (60.0)	43 (59.7)	41 (56.9)	0.839
Predominantly solid	29 (24.2)	19 (65.5)	16 (55.2)	0.375
Predominantly cystic	17 (14.2)	6 (35.3)	7 (41.2)	1.000
Cyst	2 (1.7)	1 (50.0)	0 (0.0)	-
Internal vascularity				
Yes	56 (46.7)	36 (64.3)	34 (60.7)	0.815
No	64 (53.3)	33 (51.6)	30 (46.9)	0.629
Calcifications				
Microcalcification	33 (27.5)	30 (60.6)	17 (51.5)	0.508
Macrocalcification	17 (14.2)	8 (47.1)	7 (41.2)	1.000
No calcification	70 (58.3)	41 (58.6)	40 (57.1)	1.000
Needle sizes				
23G	57 (47.5)	30 (52.6)	26 (45.6)	0.481
25G	63 (52.5)	39 (61.9)	38 (60.3)	1.000

Table 3. Univariate and multivariate analysis for factor associated with adequacy of the FNA.

Factors	**Univariable analysis**		**Multivariable analysis***	
	Risk Ratio (95% CI)	***P* - value**	**Adjusted Risk Ratio (95% CI)**	***P* - value**
Group				
CS	1.08 (0.91, 1.28)	0.398	1.08 (0.92, 1.26)	0.371
LBS	Reference		Reference	
Nodule characteristics				
Size				
< 1 cm	Reference			
1- 2 cm	1.18 (0.78, 1.78)	0.423		
> 2 cm	1.21 (0.81, 1.80)	0.358		
Solidity				
Solid	1.53 (0.90, 2.59)	0.118	1.51 (0.90, 2.54)	0.116
Predominantly solid	1.58 (0.90, 2.77)	0.111	1.68 (0.99, 2.86)	0.056
Predominantly cystic	Reference		Reference	
Cyst		0.687	0.83 (0.12, 5.64)	0.853
Internal vascularity				
Yes	1.27 (0.97, 1.66)	0.081	1.30 (1.01, 1.67)	0.038
No	Reference		Reference	
Calcifications				
Microcalcification	0.97 (0.72, 1.31)	0.839		
Macrocalcification	0.76 (0.47, 1.23)	0.267		
No calcification	Reference			
Needle sizes				
23G	Reference		Reference	
25G	1.24 (0.94, 1.64)	0.121	1.39 (1.07, 1.79)	0.013

* Adjusted for variable with $P < 0.15$ from univariable analysis

Intra-observer reliability

Intra-observer reliability for cytological adequacy in the randomly selected 20 cases from each method was in perfect agreement with CS (kappa = 1.0) and almost in perfect agreement with LBC (kappa = 0.90).

Discussion

In this prospective study, adequacy rate in CS was comparable with the adequacy rate in LBC (ThinPrep). Although the difference was not statistically significant, this finding was more obvious in the comparative study by Cochand-Priollet B, *et al.* showing a higher adequacy rate in CS than LBC using ThinPrep (92.0% vs. 78.0%), and also in the prospective study by Liu X, *et al*. which showed significantly higher adequacy rate in CS than LBC using SurePath (63.8% vs. 57.2%, $P = 0.04$). [9, 12] This finding is contradictory to our hypothesis and the previous data presuming that LBC has the benefit of lowering the inadequacy. As well as decreasing the bloody smear, LBC also uses automated processes to prepare only one slide for each nodule, which is simpler for the interventionist to prepare and for the cytologist to read compared with smearing conventional glass slides, which has person-to-person variation.[13] Our negative result may be attributable to two factors. The first is the cytomorphological change of LBC that can lessen the adequacy. Several studies found that there was diminished colloid in LBC slides and dispersed lymphocytes, rendering the diagnosis of sample adequacy and diagnosis of Hashimoto's thyroiditis more difficult. [10, 12, 14] The second factor is the early experience of using LBC in our institution by the interventionists and our personnel involving in FNA procedures and LBC processing. This is a potential explanation for the suboptimal adequacy of LBC in our study because the application of LBC has a learning curve, as recognised in the meta-analysis by Chong Y, *et al.,* which demonstrated a trend for the better adequacy of LBC after introduction of the system from 2000 to 2013.[1] However, after combining our results with the current literature review, we can infer that LBC superiority over CS is still uncertain due to the lack of support by a well-controlled prospective study. [1, 6, 9, 12]

Considering the factor that affects the adequacy of FNA in both groups, we found that the presence of internal vascularity on ultrasound is a potential predictor of sample adequacy. This result was in contrast to the widely accepted idea that blood contamination is an important cause of non-diagnostic cytology. [1, 15] In addition, the study by de Koster EJ, *et al.* using ThinPrep in thyroid FNA showed that increased intra-nodular vascularity on US was associated with a significantly higher non-diagnostic rate. [15] Conversely, Grani G, *et al.* reviewed a large number of thyroid FNA (3,279 cases) and found that increased vascularity on ultrasound did not significantly increase non-diagnostic results and could represent cellular proliferation of the nodule rather than an increased chance of blood contamination. [5] Nevertheless, data about this issue is still limited.

Regarding the adequacy in other nodule characteristics, the study by Liu X, *et al.* showed the significantly better performance of CS over LBC, especially in solid nodules with an adequacy of 78.2% and 68.0%, respectively and when excluding nodules less than 1 cm. [9] In addition, Lee YJ, *et al.* retrospectively studied 112 thyroid nodules using ThinPrep in FNA, showing the high prevalence of inadequacy in predominantly cystic nodules.[13] However, our study did not show any significant difference in sample adequacy regarding the size, solidity and calcification of the nodule.

Regarding the needle size for FNA, the meta-analysis by Moss WJ, *et al.* using CS in thyroid FNA and the randomised study comparing needle sizes in LBC by Jung SJ, *et al.* revealed no significant difference in sample adequacy among the needle sizes ranging from 21 - 27G. [16, 17] Interestingly, this study found that the use of 25G needle significantly associated with more adequacy of thyroid FNA as compared with 23G needle. Thus, with the potential less pain from smaller needle size, the use of a 25G needle may be recommended rather than a 23G needle. [16, 18]

There are several limitations to our study. First, there was a relatively low adequacy of FNA in both methods (57.5% in CS and 53.3% in LBC) compared with the adequacy rate reported in the literature (63.0 – 98.0%).[1, 2] The adequacy rate in our study is similar to that in a retrospective study conducted in the Department of Pathology in the same hospital by Limlunjakorn P. in 2017, which was 52.4%[19] This was probably due to the suboptimal FNA skill of operators, who were mostly the training fellows (98.0%) in our study. This low adequacy rate not only lessened the true difference of adequacy between the two methods, but led to repeated FNA, unnecessary surgery and additional costs for the healthcare

system. This issue was addressed and fixed by a special program for trainees in the Department of Pathology.[20] However, more efforts are needed to improve the diagnostic rate of FNA, including further training of interventional radiology fellows and collaboration between the Department of Radiology and the Department of Pathology to enhance specimen handling and cytological processes in our hospital to the highest quality. Second, there is potential bias in sample separation. Our study protocol was designed to solve the problem addressed in the study by Liu X, *et al*. by randomizing the sequence of sample preparation (either CS or LBC first) to balance the effect of bloody smears in the latter needle passes between the two groups.[9] However, the number of needle passes and the separation of the sample into halves still relied on the operator's judgement, so we could not blind the method being sampled to the operators. Therefore, it is possible that there could be a disproportionate amount of sample for each group in some cases. Third, we did not record some details of ultrasonographic characteristics of the nodules, such as echogenicity and margins, which could correlate with the adequacy of FNA.

A future study that uses a larger number of subjects by a team with high experience in using LBC for thyroid FNA is suggested, with particular focus on nodules with internal vascularity.

Conclusion

Our prospective study did not show the superiority of LBC over CS in reducing the sample inadequacy of thyroid FNA. The presence of internal vascularity on ultrasound was associated with sample adequacy. The use of a 25G needle should be preferred over a 23G needle for thyroid FNA.

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Conflict of interest

The authors, hereby, declare no conflict of interest.

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