

Original Article

Adequacy rate comparison between liquid-based cytology using SurePath versus conventional smears in detecting thyroid malignancies

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Abstract: Background: Controversy exists about the diagnostic value of liquid-based cytology (LBC) compared to conventional smears (CS). Most prior studies of LBC were performed using ThinPrep system. Few studies have ever compared the adequacy rate of SurePath with conventional smears. Methods: We performed a prospective comparison of LBC using SurePath with CS in 304 thyroid nodules. Four needle sticks constituted a single nodule FNA, with 2 passes used for CS while the other 2 passes were used for SurePath. Cytopathologists separately read all samples, and all slides were reported using the Bethesda system for reporting thyroid cytology. The adequacy rate was compared between the CS and SurePath groups. Results: The adequacy rate for all solid nodules was 78.2% in CS group, significantly higher than 68.0% in the SurePath group ($P=0.006$). No significant difference was seen for mixed or cystic nodules. The adequacy rate using a combination of CS and SurePath in solid nodules was 86.4%, significantly higher than 78.2% in CS group ($P<0.001$). When excluding nodules less than 1 cm, the adequacy rate of CS for solid nodules was 83.5%, significantly higher than 71.3% in SurePath group ($P=0.02$). The adequacy rate of combination of CS and SurePath was 91.3% for solid nodules, significantly higher than 83.5% in CS group ($P=0.04$). Conclusion: Our study showed that LBC using SurePath is not superior to conventional smears. However, a combination of both SurePath and CS may yield the most favorable adequacy rate compared to either process separately.

Keywords: Liquid-based cytology, thyroid cytology, conventional smear, adequacy rate, comparison, thyroid nodule, thyroid malignancy

Introduction

Thyroid nodules are increasingly common [1] and require further evaluation to rule out malignancy as suggested by various guidelines [2-6]. Cytological analysis of fine-needle aspiration (FNA) material is the primary and cost-effective modality for initial evaluation of a clinically relevant thyroid nodule [2-5, 7, 8]. Conventional smear (CS) for cytology diagnosis has been the main method for evaluation.

Since its introduction in 1996, liquid based cytology (LBC) has shown its advantages over conventional smears. Liquid-base preparations (LBP) were consistently devoid of obscuring elements, and the cells were adequately preserved and evenly dispersed [9]. However,

other studies showed that LBC has its own disadvantages, for example, higher non-adequacy rate [10], artifact which might lead to diagnostic pitfalls [9], missing of background factor which is essential clue for certain cases [11].

Controversies still exist about whether thyroid fine-needle aspirates (FNAs) should be processed solely with conventional smears or LBC [12]. Each has its own advantages and disadvantages as described. As a result, the combination of conventional smear with liquid-based preparations is often suggested [11] if LBC is available.

However, previous comparison studies have certain limitations. They were either small sample sized, or non-head-to-head comparison, or non-prospective. Most of the previous compari-

sons studies were using ThinPrep system, few has used another FDA-approved liquid based preparations, the SurePath system [13]. Nagarajan reported significantly higher percentage of non-adequacy rate was found in LBP compared to conventional smears in a very large sample [10]. However, different nodules were included from the two different groups. Although the age, sex and nodule size were comparable between LBP and CS groups, the component and calcification within the nodule were not mentioned which were very important factors influencing the adequacy rate [14-16]. Additionally due to retrospective nature of this study, the result might not be applicable to other centers. Tripathy directly compared the diagnostic accuracy of LBP versus CS from the same sample [11]. However, only 18 cases of thyroid nodule were recruited in this study. Until now only two studies described LBP using SurePath system for thyroid cytology [13, 17]. One study compared the efficacy of SurePath vs. conventional smears in the FNA of thyroid nodules [17]. However, they used the remaining sample in the needle after direct smear for SurePath slides. This might constitute a source of inconsistency, since the proportion of remaining material in the total sample varied from time to time after making smears. To the best of our knowledge, no study has ever compared the LBP using SurePath and CS concerning the adequacy rate in separate sample from the same thyroid lesion after the publication of the Bethesda system in 2009 [18].

To further illustrate this issue, we implemented a direct comparison between LBP using SurePath system and conventional smears in a large sample from China.

Methods

Patients

We recruited 304 thyroid FNA specimens from 304 patients prospectively from Sep 1st 2014 to Jun 31st 2015 at the Department of Endocrinology in First Affiliated Hospital of Nanjing Medical University. The ultrasound-guided FNA biopsy was performed by an experienced radiologist (L.W.) and one of the endocrinologists (X.L., Y.C., Z.W., D.C., H.F., L.J.). All the patients signed the written form consent before the procedure of thyroid FNA.

FNA procedure

The patient was placed in a supine position with a rolled towel behind the lower cervical spine to extend the neck. After the lesion is localized, the overlying skin is cleansed with 75% ethyl alcohol. A high-resolution (6-18 MHz, Esaote MYLAB 60 system, Italy) linear-array transducer, with a sterile cover placed over its head, was used for ultrasound examination and real-time guiding. The component of nodules was documented as hypoechogenicity (hypo), solid, cystic and mixed. The hypoechogenic area was diffusely hypoechogenic area under the ultrasound mimicking a nodule however the border was obscure which made precise measurement impractical. This situation was mostly consistent with subacute thyroiditis or Hashimoto's thyroiditis. All the ultrasound was performed by one radiologist who has more than 10 year experience of performing thyroid ultrasound (L.W.).

We performed FNA using four passes of 25 G needle (0.5 mm*38 mm, Becton Dickinson) attached to 5 mL syringe (Becton Dickinson) with local anesthesia of 2% lidocaine prior to the FNA as previously described [19]. The transducer was placed directly over the lesion. The patient was instructed not to swallow or speak during the insertion of the needle. A freehand biopsy technique is used, and the syringe attached to the needle is placed just above the transducer. The needle was introduced parallel or perpendicular to the transducer according to the location of the nodule and the preference of the operator, and the needle tip or sheath was carefully monitored during the procedure. When the needle reached the target, the aspiration was performed. During the procedure, all needle movements were continuously visualized in real time. All the FNAs were done with the guidance of ultrasound. Smears from the first two passes were prepared using the conventional method. The slides were immediately immersed in 95% alcohol. The needles from the next two passes were rinsed in a tube containing 15 mL of preservative solution (CytoRich® Red Preservative Fluid) and then processed via PrepStain® Slide Processor (TriPath Imaging, Inc., 780 Plantation Drive, Burlington, North Carolina, 27215, USA) according to the manufacturer's instruction. One slide was made by the SurePath method. The SurePath and direct smear slides were stained with Papanicolaou stain.

Table 1. Baseline characteristics of the patients

	Hypo*	Solid	Mixed	Cystic	Total
Number (%)	7 (2.3%)	206 (67.8%)	52 (17.1%)	39 (12.8%)	304
Age (ys)	47.0±9.8	46.6±13.8	50.0±12.5	51.7±14.8	47.8±13.7
Gender					
Male, n (%)	0	31 (15.0%)	15 (28.8%)	10 (25.6%)	56 (18.4%)
Female, n (%)	7 (100%)	175 (85.0%)	37 (71.2%)	29 (74.4%)	248 (81.6%)
Size					
Mean size (cm)	NA	1.4±1.0	2.0±0.9	2.7±1.1	1.7±1.1
≥1 cm n (%)	NA	115 (55.8%)	44 (84.6%)	36 (92.3%)	195 (64.1%)

*Hypo was short for hypoechogenicity area which was diffusely hypoechogenic area under the ultrasound mostly consistent with subacute thyroiditis or Hashimoto's thyroiditis. For these cases, the borders of these "nodules" were very obscure and precise measurement was impractical. Abbreviations: NA, not available.

Cytopathology preparation and interpretation

All the samples were reported using the Bethesda System as follows: Nondiagnostic (ND), Benign (B), Atypia of Undetermined Significance (AUS), Suspicious for Follicular Neoplasm (FOL), Suspicious for malignancy (SUS) and Malignant (M) [18]. Notably, the ND rate of each group was the primary concern of current study. For a thyroid FNA specimen to be satisfactory for evaluation (and benign), at least 6 groups of benign follicular cells are required, each group composed of at least 10 cells. Inadequate samples are reported as ND. We also appreciate several exceptions to the numeric requirement of benign follicular cells when abundant colloid or any atypia is presented [18]. A sparsely cellular specimen with abundant colloid is considered as B. Whenever there is atypia, the sample is not designated as ND. All the slides were reviewed by attending cytopathologist (R.R.) first then confirmed by chief cytopathologist (Y.W. or Q.Y.). If any discrepancy occurred, final decision was made after the discussion with executive chief cytopathologist (Z.Z.). Cytological diagnosis was made only on the basis of each slide.

Final diagnosis was made as non-diagnostic when both CS and SP diagnosis were non-diagnostic. If one of the two diagnoses was non-diagnostic, the final diagnosis was made according to the one with diagnostic cytology. If both were diagnostic but different, final diagnosis was made according to the one with higher risk of malignancy recommended by the Bethesda system [18], as this was most likely to inform clinical decision making in the real-world context.

Statistical analysis

Quantitative data were shown as mean ± SD, whereas numbers and percentage were provided for qualitative data. Quantitative data were compared using independent samples T-test. Percentages were compared using the χ^2 test. All tests were 2-sided, and a *P* value <0.05 was considered

statistically significant. Adequacy rate comparisons were made using McNemar Test. Statistical analyses were performed with SPSS software, version 13.0 for Windows (SPSS Inc, Chicago, IL, USA).

This study protocol was reviewed and approved by the Institutional Review Board (IRB) of the First Affiliated Hospital of Nanjing Medical University. It was approved by the IRB for analysis.

Results

Baseline characteristics of the patients and nodules

We recruited 304 nodules from 304 patients with average age at 47.8±13.7 years old and 81.6% being female. The mean size of the nodules was 1.7±1.1 cm with 67.8% being solid nodules (**Table 1**).

Final cytological diagnosis of the nodules with different components

The adequacy rate for all nodules in current study was 74.7% while 8.9% of the nodules were diagnosed as M and 13.2% were SUS. For nodules with solid component the adequacy rate was 86.4% and 12.6% were M and 19.4% were SUS. The non-diagnostic rate increased from 13.6% in nodules with solid component to 69.2% in nodules with cystic component (**Table 2**).

Adequacy rate comparison between conventional smear, SurePath and combined methods

Detailed distribution of cytology diagnosis for both conventional smears and SurePath were

Table 2. Nodule component and final cytological diagnosis

	ND	B	AUS	FOL	SUS	M	Total
Hypo*	5 (71.4%)	1 (14.3%)	1 (14.3%)	0	0	0	7
Solid	28 (13.6%)	51 (24.8%)	58 (28.2%)	3 (1.5%)	40 (19.4%)	26 (12.6%)	206
Mixed	17 (32.7%)	26 (50.0%)	8 (15.4%)	0	0	1 (1.9%)	52
Cystic	27 (69.2%)	11 (28.2%)	1 (2.6%)	0	0	0	39
Total	77 (25.3%)	89 (29.3)	68 (22.4%)	3 (1.0%)	40 (13.2%)	27 (8.9%)	304

*Hypo was short for hypoechogenicity area which was diffusely hypoechogenic area under the ultrasound mostly consistent with subacute thyroiditis or Hashimoto's thyroiditis. For these cases, the borders of these "nodules" were very obscure and precise measurement was impractical. Abbreviations: ND, Non-diagnostic; B, Benign; AUS, Atypia of Undetermined Significance or Follicular Lesion of Undetermined Significance; FOL, Follicular Neoplasm or Suspicious for a Follicular Neoplasm; SUS, Suspicious for Malignancy; M, Malignant.

Table 3. Cytology Distribution among BD SurePath and conventional smear

		BD SurePath Cytology						
		ND	B	AUS	FOL	SUS	M	Total
Smear Cytology	ND	77	19	13	0	1	0	110
	B	30	38	2	0	1	0	71
	AUS	19	2	34	0	5	1	61
	FOL	1	0	0	2	1	0	4
	SUS	2	0	7	0	23	3	35
	M	1	0	1	0	11	10	23
	Total	130	59	57	2	42	14	304

Abbreviations: ND, Non-diagnostic; B, Benign; AUS, Atypia of Undetermined Significance or Follicular Lesion of Undetermined Significance; FOL, Follicular Neoplasm or Suspicious for a Follicular Neoplasm; SUS, Suspicious for Malignancy; M, Malignant.

showed in **Table 3**. As shown, discrepancy existed between conventional smear and SurePath cytology. For all nodules, adequacy rate for conventional smears was 63.8% significantly higher than in 57.2% in SurePath group. This was mostly attributable to solid nodules as the adequacy rate for solid nodules was 78.2% in CS group significantly higher than 68% in BD group, while for mixed, cystic nodules and hypo-echoic areas there was no significantly difference between BD and CS groups. When comparing the final cytology diagnosis with conventional smear, the final diagnosis rates were significantly higher than in convention smear group for all nodules, solid nodules, mixed nodules and cystic nodules (**Table 4**).

When excluding nodules less than 1 cm, no significant difference was found for all nodules using BD and CS method. For solid nodules, however, the adequacy rate was significantly higher in CS group than in BD group (**Table 5**). When comparing the final adequacy rate with

CS diagnosis, similar trend was found as for nodules with various components (**Table 5**).

Discussion

Our prospective study showed that although less adequacy rate was found in the SurePath group compared to CS group especially for solid nodules ≥ 1 cm, combination of both methods would increase the adequacy rate even further on the basis of each method alone.

Non-diagnostic rate in FNA has been a dilemma and an area of controversy. The exact frequency and mechanism of non-diagnostic cytology for thyroid FNA is unknown. Brito reported that 39% of thyroid experts estimated the frequency of non-diagnostic USFNA to be above 10% [16]. At first glance, the non-diagnostic rate in our study was 25.3% similar to the rate found by Geers and Bourgain [13] using SurePath as well, higher than those using ThinPrep [20]. However, it is well documented that the greatest risk factor predicting a non-diagnostic aspirate is the nodule's cystic content [14, 15]. In current study, final adequacy rate in solid nodules with diameter ≥ 1 cm was as high as 91.3%, while for mixed and cystic nodules in the same size group the adequacy rates were only 70.5% and 30.6%. All these data confirmed the results from previous studies suggesting the adequacy rate was closely related to the nodule composition. In another study by Guo et al, they reported that the non-diagnostic rate was as low as 2%, however, the malignant rate was as high as 86.9% suggesting a highly select sample [21]. Yassa reported a total of 2587 sequential patients with thyroid nodules in 10 years, how-

Table 4. Adequacy rate comparison between BD SurePath and conventional smears (CS), Conventional smears and final diagnosis for all nodules

	n	BD	CS	Final	P ¹	P ²
All	304	57.20%	63.80%	74.70%	0.04	<0.001
Solid	206	68.00%	78.20%	86.40%	0.006	<0.001
Mixed	52	42.30%	48.10%	67.30%	0.678	0.002
Cystic	39	25.60%	15.40%	30.80%	0.289	0.031
Hypo	7	28.60%	28.60%	28.60%	1	1.00%

Note: P¹ for adequacy rate comparison between BD SurePath and conventional smears; P² for adequacy rate comparison between final cytology diagnosis and conventional smears; Abbreviations: BD, BD SurePath; CS, conventional smear.

Table 5. Adequacy rate comparison between BD SurePath and conventional smears (CS), Conventional smears and final diagnosis for nodules with size ≥1 cm

	n	BD	CS	Final	P ¹	P ²
All	195	58.50%	62.60%	75.40%	0.358	<0.001
Solid	115	71.30%	83.50%	91.30%	0.02	0.04
Mixed	44	50.00%	47.70%	70.50%	1	0.002
Cystic	36	27.80%	13.90%	30.60%	0.125	0.031

Note: P¹ for adequacy rate comparison between BD SurePath and conventional smears; P² for adequacy rate comparison between Final cytology diagnosis and conventional smears; Abbreviations: BD, BD SurePath; CS, conventional smear.

ever only 373 of 2587 patients (14.4%) had thyroid cancer that measured ≥1 cm in greatest dimension, as determined by pathologic examination [20]. In the report by Alexander [14], the non-diagnostic rate was 13% for all nodules. In sub-analysis the initial non-diagnostic rate for solid nodules was 8% which was similar to 8.7% for solid nodule with size ≥1 cm in our study. All these data suggest that when reporting the adequacy rate of a certain center, percentage of the nodules with different composition is needed. Otherwise the adequacy rate is not comparable. Other factors, for example, coarse or rim calcifications as well as proximity to important anatomic structures (such as the carotid artery or trachea) can limit the ability of the practitioner to obtain adequate tissue [15]. In some cases, no cause can be identified [15]. FNA volume and technique may also influence the adequacy rate. Our previous study showed that multiple passes using 25-gauge needle is superior to 22-gauge needle in obtaining enough sample for cytology evaluation [19].

Controversies exist regarding the lower size cut-off for thyroid FNA. The American Association

of Clinical Endocrinologists (AACE) and Korean Society of Thyroid Radiology (KSTR) guidelines [3, 5] recommend that nodules of any size with suspicious features undergo biopsy, whereas the ATA guidelines do not recommend biopsy of subcentimeter nodules unless the patient has a high-risk history [2]. In current study the adequacy rate for nodules <1 cm was 74.2% comparable to 75.4% in the nodules with size ≥1 cm (P=0.886) suggesting that the size had no major impact on the adequacy rate confirming previous result [21]. However, further study is needed regarding the clinical significance of performing FNA for nodules with size less than 1 cm.

ATA guideline for thyroid nodules suggests repeating USFNA of these cytologically non-diagnostic nodules [6]. However, there is no specific recommendation or evidence on how and when this re-aspiration should be done to avoid the potential possibility for another non-diagnostic cytology diagnosis. A recent study from Brito [16] revealed that the most common approaches to increase the diagnostic yield were (1) use of suction with USFNA

and (2) changing the targeted area of biopsy within the nodule. Few considered the patients' preferences as an important driver for the management of non-diagnostic USFNA. Finally, a molecular test for bypassing non-diagnostic USFNA was regarded as the most needed strategy for future research. How to improve the adequacy rate for thyroid FNA has been a major area for further research. Klooker demonstrated that a significantly better diagnostic performance was achieved by using the screw needle compared to the conventional fine needle in cytology of thyroid nodules [22]. However, sampling technique using LBP has not been mentioned in this study. As we may draw from our current study that including a combined method using CS and SP might be one of the options for decreasing the non-diagnostic rate.

We acknowledge limitations to our study. Ideally the adequacy rate for SurePath might be higher than that in CS group, since fewer cells were expected to be left in the residual of the needle base after rinsing compared to being expelled onto the smear glass. Most possible explanation might be that the SurePath samples were

performed after the conventional smear. There might be traditionally more bruising/bleeding on those later sticks which could compromise adequacy. Also, some patients are in pain, and the latter sticks are therefore shorter. A cross over design is needed to further eliminate the potential impact. Adequacy rate comparisons were made only between cytological diagnosis with different sample preparation methods instead of cytology diagnosis and histology diagnosis which might be the golden standard for comparison. The main purpose of current study was to compare the adequacy rate between different methods. The comparison with histology might be less demanded. However, we do believe that long term follow-up for surgical pathology is warrant for further illustrating this issue.

In conclusion, our study showed that LBC using SurePath is not superior to conventional smears. However, combined methods using both methods may further increase the adequacy rate for thyroid FNA than each method alone.

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Disclosure of conflict of interest

None.

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