

Diagnostic Value of Liquid-Based (Liqui-PREP) Preparations and Interobserver Reproducibility in Fine Needle Aspiration Cytology of the Nodular Thyroid Lesions

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The aim of this study is to define the diagnostic role of Liqui-Prep (LP) technique for the diagnosis of thyroid lesions and to assess interobserver variabilities. In all, 98 thyroid FNA (fine needle aspiration) cytology samples from 83 patients for preoperative evaluation of thyroid nodules, prepared by the LP, were examined. The LP slides were independently evaluated by three pathologists and they were classified into the five categories according to the Bethesda system. The histopathologic diagnoses were grouped as follows: benign, follicular neoplasm, and malignant. Agreements between LP and histopathologic diagnoses were investigated using kappa values. Marginal homogeneity and kappa tests were used for statistical analysis for the evaluation of the agreement between the pathologists and the interobserver variability of the thyroid aspiration cytology results. The presence of nucleoli, nuclear grooving, overlapping, intranuclear inclusion, and nuclear irregularity were recorded and scored on each case based on LP slide review. Concurrences between LP and the histologic diagnoses for the three pathologists were almost perfect ($k = 0.798$; $k = 0.826$; $k = 0.880$). When the observers were paired there was no significant difference from the diagnostic point of view. Interobserver agreement among the three pathologists was moderate. Initially diagnostic difficulties may occur because of the inadequate experience of the pathologists concerning the evaluation of the cytologic changes associ-

ated with this new technique. Liqui-Prep technique is useful for the cytologic diagnosis of the thyroid nodules. The nuclear irregularity was the most essential feature for the differentiation of malignant lesions from follicular neoplasm by the LP method. Diagn. Cytopathol. 2011;00:000–000. © 2011 Wiley-Liss, Inc.

Key Words: Liqui-PREP; liquid-based cytology; thyroid; fine needle aspiration; interobserver variability

FNA plays an essential role for the preoperative diagnosis of thyroid lesions.¹ The optimal diagnostic strategy aims to avoid thyroidectomy in patients with benign thyroid disease. Many technical approaches have been devised to improve the quality of thyroid FNA cytology. During the past 10 years, liquid-based cytologic preparations (LBP) has been carried out for a variety of cytologic samples, including thyroid FNA.^{2–4} The LBP provides optimal cellularity for evaluation purposes by adequately and rapidly fixing specimens. This technique also decreases the number of red blood cells, inflammatory cells, and mucus. The Liqui-PREP (LP) preparation system, a new LBP method, was recently introduced on the basis that it is more cost-effective than the existing LBP methods. Furthermore, very little is known about the LP results of thyroid FNA diagnosis or how it compares with the histopathologic diagnosis of thyroid nodular lesions.⁵ The aim of this study was to assess the role of the LP technique, to investigate its accuracy for the diagnosis of thyroid lesions, and to evaluate the interobserver variability of the LP method, using direct sampling from preoperatively aspirated nodules which were examined for frozen section.

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Materials and Methods

The study was performed during a 2-year period of April 2008 to March 2010. It includes 98 FNAs from a total of 83 patients (15 men, 68 women, aged 14–81 years; average age 48.81 years) performed on surgically resected thyroid specimens which were sent to be evaluated for frozen section on Deutsches Krankenhaus Universal Hospitals Group Taksim Pathology laboratory. FNA was done for nodular thyroid lesions. The FNAs were taken with a 25 gauge needle. Three passes were performed on each nodule. The materials were mixed with the Liqui-PREP™ (LGM-International, Fort Lauderdale, FL) preservative solution which contained ethanol as a fixative. At least 1 hour was a requisite for the preservative solution to be effective. The samples were then centrifuged for 10 min. Supernatants were decanted and cellular pellet sizes were estimated. Pellets were then suspended in the encapsulating (Liqui-PREP cellular base) reagent. Cell density was controlled by estimating the ratio of cell pellet to Cell Base reagent added to each sample. Aliquots (50 µl) of each homogeneous suspension were transferred to clean microscope slides and spread into circles. After drying, slides were stained with Hematoxylin-Eosin and coverslipped. LP slides were examined blindly by three independent pathologists without clinical information. All samples were satisfactory for the evaluation. The thyroid FNA samples prepared by LP were categorized into one of five groups according to the Bethesda reporting system as benign, follicular lesion of undetermined significance/atypia of undetermined significance, follicular neoplasm/suspicious for follicular neoplasm, suspicious for malignancy and malignant.^{1,6} For statistical analysis, these five categories were assigned numbers from 1 to 5. The histological result of thyroidectomy sampling was considered as the final diagnosis. The histologic diagnosis was classified into three groups for statistical analysis as: benign diseases [nodular goiter (multifocal or solitary adenomatous hyperplasia), nodules in Graves' disease, lymphocytic thyroiditis] was one; follicular neoplasms (follicular adenoma, minimally invasive follicular carcinoma) were two; malignant neoplasms (papillary carcinoma, medullary carcinoma, anaplastic carcinoma) were three.

Results of the cytological and the histological diagnosis were compared in order to evaluate the role of Liqui-PREP liquid-based cytology technique. Some follicular adenomas (macrofollicular type) diagnosed as benign on LP slides were accepted as compatible with the histologic diagnosis.⁶ Lesions with undetermined significance/atypia of undetermined significance and suspicious for malignancy diagnosis were excluded. Kappa statistic was calculated to adjust for the agreement between the original histologic diagnosis and each LP diagnosis by the three pathologists. The agreements were considered fair, moderate, and substantial for kappa values <0.40, 0.41–0.60,

and 0.61–0.75 respectively. A kappa value over 0.75 was considered as almost perfect agreement. Agreement between the pathologists was evaluated with the marginal homogeneity test as there were more than two pathologists. A *P* value of greater than 0.05 was considered that there was no significant difference between the agreements of the two pathologists. Then kappa test was used to assess the compatibility between the two pathologists and the statistical significance. For statistical analysis, SPSS packet programme version 17.0 was used.

The cytologic evaluation was performed according to the specific features of the nuclear changes and they were scored as follows: small nucleoli, 1 or conspicuous nucleoli, 2; regular nuclear contours were assessed according to the increasing volume 1, 2, 3, 4, or absent 0; irregular nuclear contour was evaluated in the same way respectively 1, 2, 3, 4, or absent 0. If the nuclear grooving, intranuclear inclusion, and overlapping were present they were classified as 1 and as 0 when absent. The correlation between diagnosis and nuclear changes were assessed with the Spearman correlation test. Overlapping, intranuclear inclusion, and groove feature correlations were evaluated with the chi-square test.

Results

Samples were obtained from 98 lesions of 83 patients. The liquid-based preparation was used for evaluation. LP slides were examined independently by three pathologists without clinical information and original pathology reports. For statistical analysis, the histopathologic diagnosis of the resected material was the gold standard. The distribution of the histopathologic diagnoses which 98 FNA samples have been obtained, are shown on Table I. The total numbers of diagnostic categories as originally determined by the three observers using LP slides are summarized in Table II. The cytologic diagnoses of the three observers using LP slides were compared with the histopathologic diagnosis of the 98 cases (Figs. 1–3). The diagnoses were almost in perfect agreement according to the kappa test, 0.798, 0.826, and 0.880, respectively, *P* = 0.000 (Table III). We also

F1-F3

Table I. The Distribution of Nodular Thyroid Lesions According to Histopathologic Diagnosis

Histopathologic diagnosis	Specific histologic diagnosis	Number of thyroidal lesions
Benign	Colloid nodule	36
Benign	Diffuse hyperplasia with nodule	2
Benign	Lymphocytic thyroiditis	13
Benign	Hyperplastic/adenomatous goiter	12
Follicular neoplasm	Follicular adenoma	11
Malignant neoplasm	Papillary carcinoma	22
Malignant neoplasm	Medullary carcinoma	1
Malignant neoplasm	Anaplastic carcinoma	1

DIAGNOSTIC VALUE OF LIQUID-BASED (Liqui-PREP) PREPARATIONS

Table II. Cytologic Results According to the Bethesda Reporting System for Liqui-PREP Slides

Cytologic diagnosis	Pathologist A	Pathologist B	Pathologist C
Benign	59 (60.2%)	57 (58.1%)	61 (62.2%)
FLUS/AUS	6 (6.1%)	6 (6.1%)	3 (3.1%)
FN	11 (11.3%)	13 (13.3%)	11 (11.2%)
Suspicious for malignancy	6 (6.1%)	5 (5.1%)	1 (1.1%)
Malignant	16 (16.3%)	17 (17.3%)	22 (22.4%)

FLUS/AUS, Follicular lesion of undetermined significance/atypia of undetermined significance; FN, Follicular neoplasm/suspicious for follicular neoplasm.

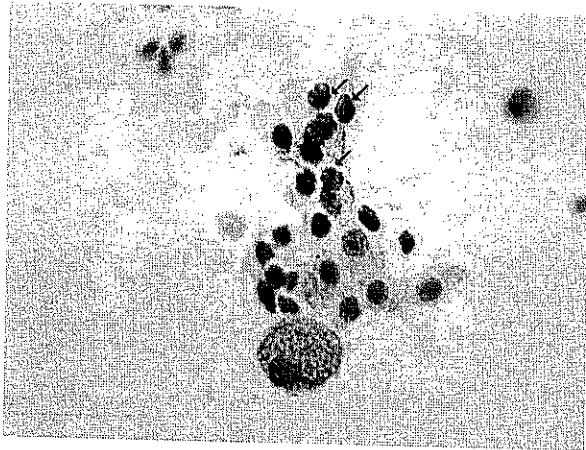


Fig. 1. Cytologic diagnosis: atypia of undetermined significance. Histopathologic diagnosis: follicular adenoma. Mildly irregular nuclear contours, nuclear indentations, and small nucleoli. The background has macrophages (H&E, $\times 1,000$). [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

compared interobserver variabilities between the reviewers for LP slides in the 35 discordant cases (Tables IV and V). When the observers were paired there was no significant difference from the diagnostic point of view ($P = 0.46$), ($P = 0.54$), ($P = 1$), respectively. Interobserver agreement between the pathologists was moderate (Table VI).

When the nuclear features are assessed by the LP technique, nuclear overlapping may be used to differentiate follicular neoplasia from benign cases according to pathologist A (82% sensitivity, 66% specificity, 30% PPV, and 95% NPV; $P < 0.006$). The presence of intranuclear inclusion can differentiate malignant cases from follicular neoplasia with a 87% sensitivity, 100% specificity, 100% PPV, and 78% NPV ($P = 0.000$). When the same groups were compared according to the pathologist C, there was a correlation between the diagnosis of malignancy and the presence of intranuclear inclusion ($P = 0.001$, $r = 0.540$ spearman; Fig. 4). According to the results of pathologist A, the presence of groove may be used to differentiate malignant cases from follicular neoplasia with a 83% sensitivity, 100% specificity, 100% PPV, and 83% NPV ($P = 0.000$ chi-square test). According to pathologist C, the presence of groove

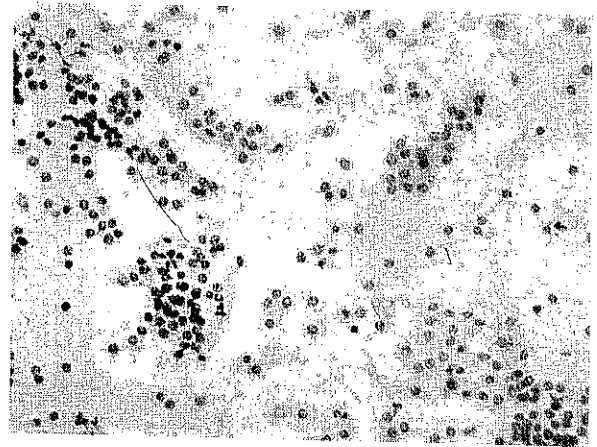


Fig. 2. Suspicious for a follicular neoplasm. The resection material revealed adenomatous hyperplasia with lymphocytic thyroiditis. Monomorphic cellular population and microfollicular pattern. Absence of colloid and regular nuclear membrane (H&E, $\times 400$). [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

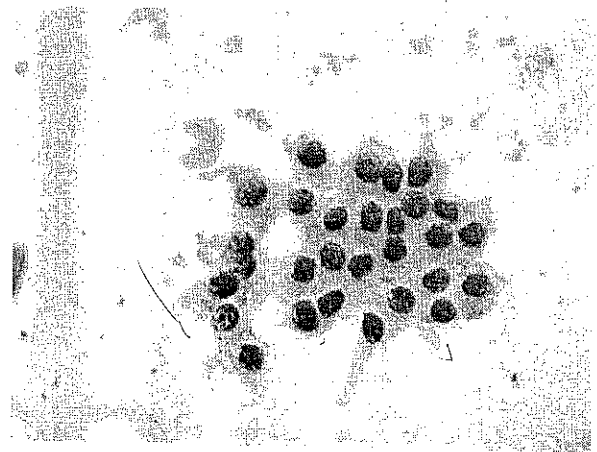


Fig. 3. Suspicious for malignancy. Histopathologic diagnosis was papillary carcinoma. Monolayered arrangement, anisokaryosis, irregular nuclear membrane, and small nucleoli (H&E, $\times 1,000$). [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

Table III. The Diagnostic Concordances of the Three Observers Using LP Slides According to the Histopathologic Diagnoses

Pathologist	Kappa value	P value
Pathologist A	0.798	0.000
Pathologist B	0.826	0.000
Pathologist C	0.880	0.000

correlated with malignancy in the same way ($P = 0.000$, $r = 0.783$ spearman; Fig. 5). Pathologist B revealed that when the benign and the follicular neoplasia cases are compared, the number of cases containing nucleolus was higher in follicular neoplasia ($P = 0.005$; $r = 0.324$ spearman). The common agreement for the evaluation of the three pathologists is

Table IV. Observer Diagnoses of 35 Discordant Cases

Case number	Histopathologic diagnosis	Pathologist A	Pathologist B	Pathologist C	Number of discordant pathologists
1	AG	1	3	1	one
2	AG	3	1	1	one
3	FA	2	1	3	two
4	FA	3	3	1	one
5	AG	3	3	3	three
6	MCa	2	4	5	two
7	LT	4	2	1	two
8	LT	2	1	1	one
9	AG	3	1	1	one
10	CN	1	1	3	one
11	CN	1	1	2	one
12	FA	3	4	2	two
13	AG	3	1	1	one
14	PCa	4	5	1	two
15	LT	1	2	1	one
16	AG	1	3	1	one
17	AG	3	1	1	one
18	FA	1	3	3	one
19	PCa	4	5	5	one
20	AG	3	3	3	three
21	AG	1	2	2	two
22	CN	1	1	3	one
23	PCa	5	4	5	one
24	AG	1	3	1	one
25	PCa	5	4	5	one
26	FA	1	1	3	two
27	LT	2	2	1	two
28	PCa	4	5	5	one
29	LT	2	1	1	one
30	PCa	4	2	5	two
31	CN	1	3	1	one
32	PCa	4	4	5	two
33	AG	1	3	3	two
34	PCa	2	3	5	two
35	PCa	1	2	4	three

AG, Adenomatous goiter; FA, Follicular adenoma; MCa, Medullary carcinoma; LT, Lymphocytic thyroiditis; CN, Colloid nodule; PCa, Papillary carcinoma.

The cytologic diagnosis: 1, Benign; 2, Follicular lesion of undetermined significance/atypia of undetermined significance; 3, Follicular neoplasm/suspicious for follicular neoplasm; 4, Suspicious for malignancy; 5, Malignant.

Table V. The Distribution of the Cases With Disagreement

Histopathologic diagnosis	Number of discordant cases	Percentages of discrepant diagnosis among discordant cases
Colloid nodule	4	11.42%
Lymphocytic thyroiditis	5	14.28%
Adenomatous goiter	11	31.42%
Follicular adenoma	5	14.28%
Papillary carcinoma	9	25.71%
Medullary carcinoma	1	2.85%
Anaplastic carcinoma	0	0%

that nuclear irregularity was higher in malignant tumors than follicular neoplasia ($P = 0.000$, $r = 0.806$, $r = 0.805$, $r = 0.816$, respectively). When the same groups are evaluated there is a reverse correlation between the malignancy diagnosis and the regularity of the nuclear membrane ($P = 0.000$; $r = -0.806$; $r = -0.837$; $r = -0.804$, spearman).

Table VI. Comparison of the Interobserver Variabilities of the Pathologists

Pairs of observers	All 98 cases using LP (P value)	35 discordant cases using LP (P value)
A-B	0.545 (0.000)	0.022 (0.813)
A-C	0.537 (0.000)	0.017 (0.839)
B-C	0.601 (0.000)	0.175 (0.043)

LP, Liqui Prep.

Paired kappa statistics in all 98 FNA using LP and 35 discordant cases using LP.

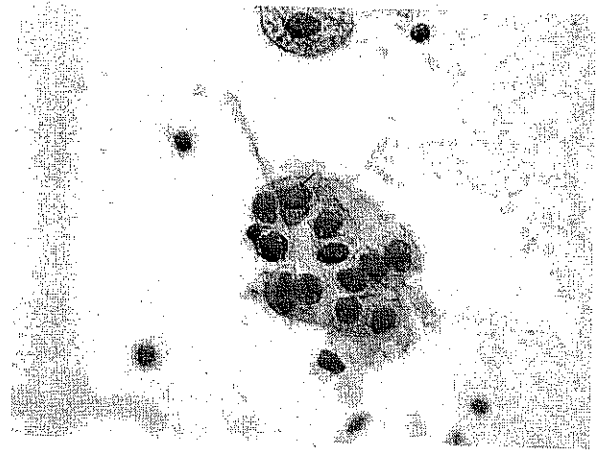


Fig. 4. Malignant. Irregular nuclear contours, small nucleoli, and intranuclear inclusion (H&E, $\times 1,000$). [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

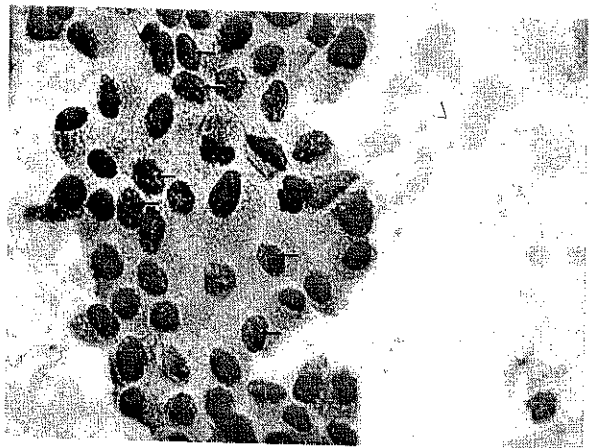


Fig. 5. Cytologic diagnosis: malignant. Histopathologic diagnosis: papillary carcinoma; crowded cluster of cells, large pleomorphic nucleus, longitudinal nuclear grooves (H&E, $\times 1,000$). [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

Discussion

Recently, LBP are replacing conventional Papanicolaou smears for cervical screening cytology in the Western

DIAGNOSTIC VALUE OF LIQUID-BASED (Liqui-PREP) PREPARATIONS

world.⁷ In some institutions, liquid-based cytology is used together with other preparation techniques such as smears and cell blocks and occasionally, liquid-based cytology is used as the sole preparation.² Advantages of LBP include faster microscopic examination of specimen, the opportunity to prepare additional slides, and to perform special stains or ancillary techniques such as immunocytochemical studies or flow cytometry.^{4,8} LBP has been attributed benefits such as increased cellularity and a lower rate of unsatisfactory or less than optimal specimens relative to conventional preparation in thyroid aspiration cytology.⁹⁻¹¹ However, its usefulness during aspiration cytology is controversial and most studies have used the Thin prep.^{2-4,8,12} Liqui-Prep (LP), the second generation of the liquid-based cytology system includes a chemical vial in which the cells were processed by a special chemical solution (cellular base) that acts as a membrane matrix. This process maintains original morphologies of the cells and, offers a monolayer of cells generating enhanced visualization. LP is claimed to provide a preparation comparable to other LBP techniques, but is more cost effective since it requires no special equipment. It has been showed to allow for an increased detection of cervical squamous cell abnormalities over the traditional conventional smear technique.^{13,14} For cervical smear, interobserver reproducibility of this technique had been reported as almost perfect.¹⁵

The results of our study suggest that LP technique may be used for the preoperative evaluation of the nodular thyroid lesions. This technique is also reliable for the differentiation of benign, follicular neoplasm, and malignant lesions (Table III). Although there appears to be individual evaluation differences, there is usually an agreement in diagnosis (Table VI). The intermediate categories such as suspicious for malignancy and follicular lesion of undetermined significance/atypia of undetermined significance in the Bethesda classification may be the cause of disagreement between the pathologists in our study. There are cases evaluated as follicular neoplasia cytologically but diagnosed as benign after the histopathologic examination of the resected specimens. Consequently, the overdiagnosis of benign disease as follicular neoplasia will at least lead to lobectomy.¹⁶

Although the LBP method can improve smear quality by reduction of the obscuring background, alterations of conventional morphological features, nuclear, and cytoplasmic details (i.e., nuclei were shrunken) induced by the different fixative solution and preparation methods have led to misdiagnoses in FNA specimens. In our study, some cases which were diagnosed by all three pathologists as follicular neoplasia were in fact adenomatous goiters. Adenomatous goiter group had the largest number of discordant cases. The decreased colloid amount and the relatively increased cellularity due to the LP technique may be the cause of this diagnostic error. The LP technique may also be the cause

for the cytologically false-positive follicular neoplasia cases which were later diagnosed as colloid nodules histologically. We have also observed the artefactual shrinkage and nuclear irregularity as reported in the previous studies.^{2,5,8,17,18} Several studies have indicated that LBP reduces the sizes of cell clusters and causes large branching sheets to fragments.^{17,18} It has been reported that diffuse colloid and lymphocytes are less obvious in LBP slides, and that intranuclear inclusions and grooves are less commonly observed.^{2,8,17,18} These morphologic differences must be recognized, and may necessitate diagnostic criteria modifications when LP is used to evaluate thyroid FNA specimens. In our study, one of the pathologists has misdiagnosed papillary carcinoma arising in the background of lymphocytic thyroiditis as a benign lesion. This finding indicates that nuclear details should be evaluated more cautiously. The LP method requires some interpretive experience in order to avoid diagnostic pitfalls.

Malignancy has been diagnosed in 10 of the 12 samples in which suspicious for malignancy was the cytologic diagnosis by the LP method. Most of the suspicious for malignancy cases in the discordant group were diagnosed as papillary carcinoma histologically because the cytological malignancy criteria were inadequate according to the observers. One case was diagnosed as follicular adenoma and the other was a lymphocytic thyroiditis. Focal cytologic atypia of the follicular epithelium has led to misdiagnosis in both cases. Diffuse lymphocytic thyroiditis was present in 6 patients out of 15 all of which had been diagnosed as follicular lesion of undetermined significance/atypia of undetermined significance. Cytologic atypia may be associated with injury and repair of the follicular epithelium. Three cases were diagnosed as papillary carcinoma after the histopathologic examination of the resection materials. Two of these cases are follicular variant of papillary carcinoma. The remaining two nodular cases were evaluated as follicular adenoma. This finding suggests that focal cytologic atypia may be present in follicular adenomas. We believe that architectural features should also be taken into account for cytologic evaluations. One nodule has been diagnosed as medullary thyroid carcinoma after the histopathologic examination of the resected material. Another observer has diagnosed the same case as suspicious for malignancy. These findings are probably due to the LP technique which may have caused morphological changes on the nuclear features leading to underdiagnosis in medullary carcinoma. The remaining three cases have been diagnosed as benign disease with cytological atypia in two.

The most important and the most specific features for papillary carcinoma which comprise the majority of the malignant cases in our study are the presence of intranuclear inclusion and groove. One of the pathologists has detected the presence of nucleolus in all of the cases that

are diagnosed as follicular neoplasia with LP. We suggest that nuclear irregularity is the crucial hallmark for the differentiation of follicular neoplasm and malignant lesions by LP technique. The presence of nucleoli and regular nucleus may appear as other outstanding features for the diagnosis of follicular neoplasm.

We suggest that initially LP should be combined with the conventional preparations to gain interpretive experience to avoid diagnostic errors. This approach may also decrease the individual diagnostic differences. The high diagnostic rate makes it a reliable technique for the evaluation of nodular thyroid lesions. The relatively low cost and the easiness of this procedure are the other advantages of this technique. Further studies with larger sample sizes are needed to demonstrate the real diagnostic significance of this method among other liquid-based techniques.

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