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The Diagnostic Significance of Liquid-Based Cytology of Thyroid Fluid for Benign and Malignant Conditions

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Abstract

Thyroid nodules are a common thyroid condition, with a detection rate of up to 20% - 50% in the general population, of which only 5% - 10% are malignant. Fine-needle aspiration cytology (FNAC) is currently the recommended preoperative screening method, while liquid-based cytology (LBC) has gained increasing application in thyroid diagnostics since 2000 due to its advantages such as a clean background and compatibility with auxiliary technologies. This article systematically reviews the value of LBC in distinguishing between benign and malignant thyroid nodules from the perspectives of methodology, morphology, diagnostic efficacy, Bethesda system grading, auxiliary techniques, limitations, and future directions, with the aim of providing evidence-based guidance for clinical and pathological professionals.

Keywords

Liquid-Based Cytology, Fine Needle Aspiration, Thyroid Nodules, Bethesda System, Diagnostic Efficacy

1. Introduction

The global prevalence of thyroid nodules has been steadily increasing annually with the widespread adoption of imaging techniques such as ultrasound. Conventional smear (CS) has a high dissatisfaction rate of 2% - 20% due to cell deformation caused by on-site blood, mucus contamination, and dry air. Additionally, the morphological overlap between follicular tumors and follicular papillary thyroid carcinoma (FVPTC) results in only moderate diagnostic consistency among observers ($\kappa = 0.45$). Liquid-based cytology (LBC) involves immediately placing

the aspirate into a fixative solution, followed by centrifugation and membrane transfer for slide preparation. This method theoretically removes excess blood and proteins, preserves nuclear membrane and chromatin details, and retains the remaining specimen for molecular testing such as immunocytochemistry (ICC), BRAF, and RAS, thereby enhancing the ability to distinguish between benign and malignant lesions [1] [2]. However, recent multicenter retrospective cohort studies have shown that LBC may lose microfollicles, giant follicle fragments, and granule bodies while removing background material, leading to a decrease in the sensitivity of follicular lesion diagnosis. The specimen rate for the <6 cell group was 3.6% higher than that of CS, particularly for micropapillary carcinoma. Additionally, there are no unified standards for ICC antibody combinations and molecular thresholds, which may introduce new observer bias [3]. Therefore, large-scale prospective studies are urgently needed to compare the sensitivity, specificity, positive predictive value, and cost-effectiveness of the two slide preparation methods across Bethesda grading categories, and to establish a multidisciplinary integrated model encompassing cytology, molecular, and imaging modalities, in order to comprehensively assess the true diagnostic significance and promotional value of LBC in thyroid FNAC [4]-[6].

2. Methodological Advances

2.1. Production Technology

Currently, commercial platforms include ThinPrep (Hologic), SurePath (BD), and the domestically produced Liqui-Prep. ThinPrep uses negative pressure filtration transfer, with cells appearing as 2 cm diameter spots on the slide; SurePath uses centrifugal sedimentation, resulting in a more uniform cell distribution. Both technologies can prepare cell blocks from the same needle wash fluid for histology and immunomarking.

2.2. Staining and Auxiliary Techniques

LBC fixative is rich in methanol or ethanol and is compatible with Papanicolaou, Diff-Quik, and HE staining; the remaining liquid is centrifuged to form cell blocks, which are then subjected to ICC or molecular testing for HBME-1, Galectin-3, CK19, CD56, BRAF V600E, and other markers, as well as BRAF, RAS, RET/PTC, and PAX8/PPARγ, significantly improving the malignant risk stratification of Bethesda III-IV nodules.

3. Morphological Feature Comparison

3.1. Background and Structure

In CS, colloid often appears as large "popcorn-like" fragments mixed with blood cells; in LBC, the background is clean, and the colloid is diluted into "small droplets," which facilitates observation of nuclear details. However, in some cases, due to colloid loss, nodular goiter may be misdiagnosed as "colloid-poor." Microfollic-

ular structures are reduced in LBC due to mechanical dispersion. The closely arranged microfollicles in traditional smears are an important basis for diagnosing follicular tumors, so the sensitivity of LBC for diagnosing follicular lesions decreases [7].

3.2. Nuclear Characteristics

LBC fixation was timely, resulting in clearer nuclear membranes, chromatin, and nuclear grooves. Studies show that the detection rate of nuclear pseudoinclusions in papillary thyroid carcinoma (PTC) is higher in LBC than in CS (78% vs 54%, $P < 0.01$), but the glassy nuclei are not prominent due to dehydration and shrinkage. Amyloid deposits in medullary carcinoma may be confused with glial cells in LBC and require differentiation using calcitonin ICC.

3.3. Immune Cell Components

In Hashimoto's thyroiditis, CS shows a large number of lymphoid follicles and eosinophil infiltration; LBC shows red blood cell lysis, separation of lymphocytes and follicular epithelium, clearer details of Hürthle cell nuclei, but reduced "infiltration" of lymphoid epithelium, making it easy to miss mild Hashimoto's background [8].

4. Evidence-Based Analysis of Diagnostic Efficacy

4.1. System Evaluation and Meta-Analysis

A meta-analysis of 42 studies involving 15,645 cases of thyroid fine-needle aspiration cytology (FNAC) showed (using the same patient cohorts):

1) Using histology as the gold standard, the overall sensitivity of LBC for diagnosing malignancy was 0.85 (95% CI 0.82 - 0.88), with a specificity of 0.96 (0.95 - 0.97); CS had a sensitivity of 0.89 (0.87 - 0.91) and a specificity of 0.95 (0.94 - 0.96).

2) Subgroup analysis: There was no statistically significant difference in sensitivity between ThinPrep and SurePath; CS showed a more pronounced advantage when the puncture operator was a cytopathologist ($P = 0.03$).

3) Unsatisfactory (Class I) rate: 8.9% for LBC and 4.2% for CS ($P < 0.001$).

4.2. Bethesda System Rating Comparison

A prospective case-control study in South Korea (n = 200) found:

1) Class II (benign) had the highest diagnostic consistency ($\kappa = 0.88$), but reduced LBC gelatin led to misclassification of "gelatin nodules" as Class III (Atypical (AUS) and Follicular Lesions of Undetermined Significance (FLUS)).

2) In Class IV (FN/SFN), CS achieved a diagnostic sensitivity of 93.6% due to abundant microfollicles, while LBC reached only 65.9%.

3) In Class VI (malignant), both methods had sensitivity >95%, but LBC's clean background made mitotic figures easier to identify, thereby improving the detection rate of medullary carcinoma and poorly differentiated carcinoma.

4.3. Diagnostic Significance of Benign Nodules

Benign nodules account for 70% - 80% of FNAC cases, and their accurate diagnosis can prevent unnecessary surgery. CS relies on abundant stroma and sheet-like follicular epithelium, while LBC, due to stroma dilution, results in a slightly reduced specificity for benign diagnosis; however, combining cell block ICC (e.g., CD56-negative, HBME-1-negative) can increase the negative predictive value for Class II nodules from 96.4% to 98.7%.

4.4. Diagnostic Significance of Malignant Nodules

PTC classic nuclear features in LBC are characterized by: elongated/oval nuclei, thickened nuclear membranes, and "coffee bean-like" nuclear grooves; dust-like chromatin and large, round pseudoinclusions within the nucleus; sparse background glial cells, with an increased detection rate of sand-like bodies (LBC 32% vs CS 19%).

For follicular papillary carcinoma, LBC shows reduced diagnostic sensitivity due to decreased microfollicles; however, when combined with BRAF V600E mutation testing, sensitivity can be improved from 71% to 92%, with specificity >98%.

5. The Added Value of Assistive Technology

5.1. Immunocytochemistry

In Bethesda III-IV lesions, the combined use of HBME-1, Galectin-3, CK19, and CD56 quadruple antibodies can increase the sensitivity of PTC diagnosis to 88% and specificity to 92%. LBC cell block sections are 3 - 4 μm thick, with good antibody penetration and low staining background.

5.2. Molecular Pathology

The detection rate of BRAF V600E mutations in PTC is approximately 60%. After DNA extraction from residual liquid from LBC, qPCR, ARMS, or NGS can be used for detection, with a minimum detection limit of 1% mutant allele burden [9]. Additionally, RAS mutations, RET/PTC rearrangements, and PAX8/PPARγ fusions can also be reliably detected in LBC, providing evidence for clinical decision-making.

6. Limitations and Challenges

6.1. Cost and Process

LBC reagent consumables cost 2 - 3 times more than CS, limiting adoption in primary care hospitals; additionally, specialized staining machines and centrifuges are required, with high training demands on technical staff.

6.2. Observer Experience Variation

Pathologists' familiarity with LBC morphology directly impacts diagnostic accuracy; a North American CAP survey found that physicians with <10 hours of LBC

training had a Class III-IV reporting rate of 26%, significantly higher than the group with >30 hours of training (14%).

7. Future Directions

7.1. Artificial Intelligence-Assisted Diagnosis

A deep learning-based LBC whole slide imaging (WSI) model has demonstrated over 90% accuracy in identifying malignancies in multicenter trials, with the potential to reduce interobserver variability.

7.2. Multi-Omics Integration

Integrating DNA/RNA extracted from LBC with proteomics and methylation profiles to construct a comprehensive "cytology-molecular-imaging" score, further reduces uncertainty in Class III lesions [10].

7.3. Standardized Training and Quality Control

It is recommended to establish a standardized thyroid LBC map and digital teaching database. Quality control indicators include:

1) Dissatisfaction rate <5%;
2) Type III rate <10%;
3) Histological concordance rate for malignant cases is >90%.

8. Conclusions

LBC is not a simple replacement for CS in distinguishing between benign and malignant thyroid lesions, but rather a complementary tool. Its advantages include: a clean background and clear nuclear details, which facilitate the identification of malignant lesions such as PTC, medullary carcinoma, and metastatic carcinoma; the remaining specimens can be used for ICC and molecular testing, improving the diagnostic accuracy of Bethesda III-IV nodules; digital slides facilitate remote consultations and AI applications.

However, limitations such as the absence of microfollicular structures, high costs, and significant training requirements hinder its widespread adoption in primary care settings. In the future, through standardized training, integration of molecular, imaging, and cytology data, and AI-assisted analysis, it is anticipated that the preoperative diagnostic pathway for thyroid nodules can be further optimized [11] [12].

Conflicts of Interest

The author declares no conflicts of interest regarding the publication of this paper.

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