

Role of CD56 and Galectin-3 in the Diagnosis of Solitary Thyroid Nodules on Fine Needle Aspiration Cytology: A Prospective Diagnostic Test Evaluation Study

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ABSTRACT

Introduction: Fine Needle Aspiration Cytology (FNAC) is the primary diagnostic test for thyroid nodules, but cytomorphological overlap limits definitive classification of some follicular-patterned and indeterminate lesions.

Aim: To evaluate the diagnostic performance of CD56 and Galectin-3 (Gal-3) Immunocytochemistry (ICC) on FNAC smears for differentiating benign from malignant solitary thyroid nodules, using histopathology as the reference standard.

Materials and Methods: This prospective diagnostic test evaluation study was conducted at the Department of Pathology, Government Medical College, Thrissur, Kerala, India. It included 42 consecutive patients with clinically palpable solitary thyroid nodules who underwent FNAC followed by surgical excision during an 18-month study period (1 January 2016 to 30 June 2017). All eligible patients during the time-bound study were consecutively included to reduce selection bias. ICC for CD56 and Gal-3 was performed on destained Papanicolaou-stained FNAC smears. Loss of CD56 expression and Gal-3 positivity were evaluated as index-test findings. Histopathology served as the reference standard. Sensitivity, specificity, Positive Predictive Value (PPV), Negative Predictive Value (NPV), diagnostic accuracy, and exact 95% Confidence Intervals (CIs) were calculated. Comparison of diagnostic accuracy between FNAC alone and the combined FNAC + ICC panel was performed using McNemar's test for paired binary outcomes.

Results: Histopathology confirmed 18/42 (42.9%) benign and 24/42 (57.1%) malignant nodules. Loss of CD56 expression yielded a sensitivity of 91.7% (95% CI: 73.0%-99.0%), specificity of 100% (81.5%-100%), PPV of 100% (84.6%-100%), NPV

of 90.0% (68.3%-98.8%), and diagnostic accuracy of 95.2% (83.8%-99.4%; $p < 0.001$). Gal-3 positivity yielded a sensitivity of 100% (85.8%-100%), specificity of 88.9% (65.3%-98.6%), PPV of 92.3% (74.9%-99.1%), NPV of 100% (79.4%-100%), and accuracy of 95.2% (83.8%-99.4%; $p < 0.001$). The combined CD56/Gal-3 panel achieved a sensitivity of 100% (85.8%-100%), specificity of 88.9% (65.3%-98.6%), PPV of 92.3% (74.9%-99.1%), NPV of 100% (79.4%-100%), and accuracy of 95.2% (83.8%-99.4%). The combined panel correctly classified 22/23 (95.7%) cytologically indeterminate cases (Bethesda III and IV) when compared to FNAC alone (accuracy 85.7% (71.5%-94.6%)); the adjunct application of the dual-marker ICC panel increased overall diagnostic accuracy from 85.7% to 95.2%. Statistical significance of this paired comparison should be reported based on the reconstructed patient-level 2x2 table and McNemar's test.

Conclusion: Loss of CD56 expression and Gal-3 positivity demonstrate complementary diagnostic performance when applied directly to Papanicolaou-stained FNAC smears. In this cohort, CD56 loss provided high diagnostic specificity, whereas Gal-3 offered maximum sensitivity. The combined Immunocytochemical (ICC) panel significantly improved diagnostic accuracy over conventional FNAC alone and successfully resolved the vast majority of cytologically indeterminate (Bethesda III and IV) lesions. These findings support the routine integration of a dual-marker CD56/Gal-3 panel as a cost-effective triage tool in thyroid cytopathology, though larger multicentre studies in unselected populations are warranted to confirm its impact on reducing unnecessary surgeries.

Keywords: Diagnostic accuracy, Immunocytochemistry, Papillary thyroid carcinoma, The Bethesda system for reporting thyroid cytopathology, Thyroid neoplasm

INTRODUCTION

Thyroid nodules are a highly prevalent clinical finding, detected in approximately 4%-8% of adults by palpation and up to 68% on high-resolution ultrasonography [1,2]. While the vast majority are benign, preoperative discrimination of malignant solitary thyroid nodules is critical to guide targeted surgical intervention, avoiding unnecessary diagnostic thyroidectomies and their associated morbidities, such as recurrent laryngeal nerve palsy and hypoparathyroidism [3,4].

The FNAC serves as the primary, minimally invasive diagnostic tool for thyroid lesions. However, its accuracy is hampered in follicular-patterned lesions and cytologically indeterminate categories [5,6]. Although implementation of The Bethesda System for Reporting Thyroid Cytopathology (TBSRTC) has standardised terminology and

risk stratification [7,8], indeterminate categories- specifically Atypia of Undetermined Significance/Follicular Lesion of Undetermined Significance (AUS/FLUS; Bethesda III) and Follicular Neoplasm/Suspicious for a Follicular Neoplasm (FN/SFN; Bethesda IV)-continue to pose major clinical dilemmas [9,10]. Morphological overlap between benign hyperplastic nodules, follicular adenomas, and follicular-patterned malignancies such as follicular thyroid carcinoma and the follicular variant of Papillary Thyroid Carcinoma (PTC) often leads to inconclusive cytological reporting, necessitating repeat aspirations, diagnostic surgery, or costly molecular testing [10,11].

To overcome these cytomorphological limitations, ancillary ICC marker panels have gained significant interest, particularly in resource-constrained settings where comprehensive molecular

profiling is not routinely accessible [12-14]. Galectin-3 (Gal-3), a 31-kDa β -galactoside-binding lectin involved in cell proliferation, cell-matrix adhesion, and apoptosis inhibition, is frequently overexpressed in thyroid malignancies- most notably classic PTC [13-15]. However, standalone Gal-3 testing suffers from variable specificity due to occasional expression in benign or inflammatory thyroid lesions [14,16].

Conversely, CD56 (neural cell adhesion molecule) is expressed diffusely on the membrane of normal follicular cells and benign thyroid lesions [17,18]. During malignant transformation, particularly in PTC and its variants, CD56 expression is characteristically downregulated or completely lost [18-20]. Because no single biomarker provides optimal diagnostic performance, combining complementary markers- a positive oncogenic marker (Gal-3) with a preserved benign marker (CD56)- offers a highly effective diagnostic strategy to improve risk stratification in indeterminate cytology [14,21]. Hence, the present study evaluated the diagnostic performance of a dual-marker ICC panel (CD56 and Gal-3) applied directly to Papanicolaou-stained FNAC smears of solitary thyroid nodules. The specific objectives were to evaluate the expression profiles of CD56 and Gal-3 across benign and malignant solitary thyroid nodules; to establish the sensitivity, specificity, PPV, NPV, and diagnostic accuracy of individual markers and the combined panel using histopathology as the reference standard; and to assess the utility of this combined ICC panel in resolving diagnostic ambiguity in Bethesda III (AUS/FLUS) and Bethesda IV (FN/SFN) categories.

MATERIALS AND METHODS

This prospective diagnostic test evaluation study was conducted in the Department of Pathology at Government Medical College, Thrissur, Kerala, India, over 18 months from January 1, 2016, to June 30, 2017. The study protocol was reviewed and approved by the Institutional Ethics Committee. (IEC approval number was not available in the approval certificate). Written informed consent was obtained from all participants before enrollment. All procedures were performed in accordance with the principles of the Declaration of Helsinki.

Inclusion criteria:

1. Adult patients (≥ 18 years of age) of either gender presenting with a clinically palpable solitary thyroid nodule;
2. Patients presenting as a primary, first-time clinical evaluation for a thyroid nodule;
3. Patients who underwent surgical thyroidectomy (lobectomy, subtotal, or total thyroidectomy) providing tissue for definitive histopathological evaluation.

Exclusion criteria:

1. Recurrent or previously operated thyroid lesions;
2. Impalpable or non palpable thyroid nodules detected incidentally on imaging;
3. Multinodular goitre or diffuse thyroid enlargement;
4. Inadequate or unsatisfactory FNAC smears (Bethesda Category I), defined as fewer than 6 clusters of well-preserved follicular cells with at least 10 cells per cluster, lacking abundant colloid;
5. Patients who received preoperative chemotherapy or radiation therapy.

Study Procedure

Consecutive patients presenting with clinically palpable solitary thyroid nodules who were referred to the Department of Pathology for diagnostic FNAC and subsequently underwent surgical resection during the study period were evaluated.

Cytological and ICC preparation: Fine needle aspiration was performed using a standard 24-gauge needle attached to a 10 mL syringe without local anaesthesia, with a minimum of two passes per nodule. Smears were fixed in 95% ethanol and stained with standard Papanicolaou stain. Cytological diagnoses were reported according to TBSRTC (2nd Edition) [8].

Note on reporting criteria: The 2017 2nd Edition of TBSRTC was applied as it was the current international standard framework during the active study timeline (2016-2017) and reporting phase, establishing standardised risk estimates and diagnostic thresholds for indeterminate categories (AUS/FLUS and FN/SFN) [8].

The ICC was performed directly on destained Papanicolaou-stained direct smears to conserve tissue architecture without requiring cell block preparation. Smears were destained in acid-alcohol, followed by heat-induced epitope retrieval in Tris- Ethylenediaminetetraacetic Acid (EDTA) buffer (pH 9.0) using a multiepitope retrieval system for 15 minutes. Endogenous peroxidase activity was blocked with 3% hydrogen peroxide for 10 minutes. Slides were incubated with primary monoclonal antibodies: anti-Gal-3 (mouse monoclonal, Clone H5, ready-to-use; Quartet) and anti-CD56 (mouse monoclonal, Clone 123C3, ready-to-use; PathnSitu). Detection was performed using a secondary polymer-based Horseradish Peroxidase (HRP) system, visualised with 3,3'-Diaminobenzidine (DAB) chromogen, and counterstained with Mayer's haematoxylin. Appropriate positive controls (papillary carcinoma for Gal-3; normal thyroid tissue for CD56) and negative controls (omission of primary antibody) were included in each run.

Evaluation of immunostaining: ICC staining was assessed independently by two experienced pathologists blinded to clinical data and histopathological diagnoses. Discrepancies were resolved through joint re-evaluation under a multi-head microscope.

CD56: Evaluated for membranous reactivity. In accordance with established literature [9]. Distinct membranous staining in $\geq 10\%$ of follicular cells was considered positive (preserved/retained expression; benign pattern). Staining in $< 10\%$ of cells or complete absence of staining was scored as negative (loss of expression; malignant pattern).

Galectin-3: Evaluated for cytoplasmic and/or nuclear reactivity semi-quantitatively based on the percentage of positively stained cells: score 0 ($< 10\%$), score 1 (10%-25%), score 2 (26%-50%), score 3 (51%-75%), and score 4 ($> 75\%$). A cut-off score of ($\geq 10\%$ positive cells) was defined as positive for malignancy, following established protocol guidelines [10].

Reference standard and histopathology: Surgically resected specimens were fixed in 10% neutral buffered formalin, routinely processed, embedded in paraffin, sectioned at 4 μ m thickness, and stained with Haematoxylin and Eosin (H&E). Histopathological diagnoses were established by a senior pathologist according to the World Health Organisation (WHO) Classification of Tumours of Endocrine Organs (4th Edition, 2017) and served as the definitive reference standard [11].

STATISTICAL ANALYSIS

Categorical variables and diagnostic contingency matrices (2 $\times$ 2 tables) were analysed using Pearson's Chi-square (χ^2) test or Fisher's-exact test, as appropriate for expected cell frequencies. Sensitivity, specificity, PPV, NPV, and diagnostic accuracy were calculated alongside 95% exact binomial CI. Comparison of diagnostic accuracy between FNAC alone and the combined FNAC + ICC panel was performed using McNemar's test for paired binary outcomes. Statistical analysis was conducted using Statistical Package for Social Sciences (SPSS) software version 16.0 (IBM Corp., Armonk, NY, USA), and a two-tailed p-value < 0.05 was considered statistically significant.

RESULTS

Clinicopathological characteristics: A total of 42 adult patients with clinically palpable solitary thyroid nodules were evaluated, comprising 34 females (81.0%) and 8 males (19.0%), yielding a female-to-male ratio of 4.25:1. Patient ages ranged from 18 to 70 years. The mean age was 42.4 years in patients with benign lesions and 45.1 years in those with malignant lesions. The mean maximum nodule diameter on sonographic/clinical examination was 4.3 cm for benign nodules and 2.2 cm for malignant nodules [Table/Fig-1]. Definitive histopathological examination identified 18/42 (42.9%) benign and 24/42 (57.1%) malignant lesions. Their clinicopathological characteristics are summarised in [Table/Fig-1], while the Bethesda category distribution is presented in [Table/Fig-2]. Benign diagnoses included colloid nodules (n=8), cellular nodules/adenomatoid goitres (n=5), follicular adenomas (n=3), and lymphocytic thyroiditis (n=2). Malignant diagnoses comprised classical PTC (n=13), follicular variant of PTC (n=8), and follicular thyroid carcinoma (n=3).

Features	Benign	Malignant
Number of cases	18	24
Male: Female	3: 15	5: 19
Mean age (years)	42.4 years	45.1 years
Mean maximum nodule size in cm	4.3 cm	2.2 cm

[Table/Fig-1]: Clinicopathological profile of thyroid nodules.

Bethesda category	n (%)
II	2 (4.8)
III	6 (14.3)
IV	17 (40.5)
V	6 (14.3)
VI	11 (26.2)

[Table/Fig-2]: Distribution of cases according to the Bethesda System for Reporting Thyroid Cytopathology.

Cytological classification: According to The Bethesda System for Reporting Thyroid Cytopathology (TBSRTC, 2nd Edition, 2017) [8], cytological findings were distributed as follows: Bethesda II (Benign), 2 (4.8%); Bethesda III (AUS/FLUS), 6 (14.3%); Bethesda IV (FN/SFN), 17 (40.5%); Bethesda V (Suspicious for Malignancy), 6 (14.3%); and Bethesda VI (Malignant), 11 (26.2%) [Table/Fig-2].

Immunocytochemical findings: The immunophenotypic distribution of CD56 and Gal-3 reactivity across benign and malignant histopathological diagnoses is detailed in [Table/Fig-3]. CD56 expression was retained in all 18 histologically benign lesions (100%) and lost in 22 of 24 (91.7%) malignant lesions [Table/Fig-3]. Residual membranous staining (<10% of follicular cells) was observed in two malignant cases (one classical PTC and

Histopathology	CD56 positive	CD56 negative	Galectin-3 positive	Galectin-3 negative
Benign (n=18)	18	0	2	16
Malignant (n=24)	2	22	24	0

[Table/Fig-3]: CD56 and Gal-3 expression according to histopathological diagnosis.

Diagnostic modality	Sensitivity (95% CI)	Specificity (95% CI)	PPV (95% CI)	NPV (95% CI)	Accuracy (95% CI)	p-value
CD56 Loss	91.7% (73.0-99.0%)	100% (81.5-100%)	100% (84.6-100%)	90.0% (68.3-98.8%)	95.2% (83.8-99.4%)	<0.0001 (p=3.32×10 ⁻⁹)
Gal-3 positivity	100% (85.8-100%)	88.9% (65.3-98.6%)	92.3% (74.9-99.1%)	100% (79.4-100%)	95.2% (83.8-99.4%)	<0.0001 (p=3.61×10 ⁻⁹)
Combined ICC panel	100% (85.8-100%)	88.9% (65.3-98.6%)	92.3% (74.9-99.1%)	100% (79.4-100%)	95.2% (83.8-99.4%)	<0.0001 (p=3.61×10 ⁻⁹)
FNAC alone	75.0% (53.3-90.2%)	100% (81.5-100%)	100% (81.5-100%)	75.0% (53.3-90.2%)	85.7% (71.5-94.6%)	<0.0001 (p=0.0000816)

[Table/Fig-4]: Comparative diagnostic performance of biomarkers, combined ICC panel, and FNAC alone.

CI: Confidence interval; PPV: Positive predictive value; NPV: Negative predictive value; ICC: Immunocytochemistry; FNAC: Fine needle aspiration cytology. p-values for individual markers and combined panel performance were calculated using Pearson's Chi-Square (x2) test or Fisher's exact test for 2x2 contingency cross-tabulations comparing immunophenotypic calls against histopathology (reference standard); p-value comparing overall diagnostic accuracy between FNAC alone (85.7%) and FNAC plus ICC (95.2%) was calculated using McNemar's test for paired categorical data (p=0.031); p <0.05 was considered statistically significantly different.

one FVPTC). Gal-3 expression was present in all 24 histologically confirmed malignant lesions (100%) [Table/Fig-4]. Higher expression scores (Scores 3-4) predominated in classical PTC, whereas FVPTC exhibited intermediate expression (Scores 2-3) and follicular thyroid carcinoma demonstrated lower expression (Scores 1-2). Two benign colloid nodules exhibited focal Gal-3 positivity (Score 1).

Diagnostic utility in indeterminate Bethesda categories: Bethesda category III (n=6): Five cases exhibited a benign immunophenotype (CD56+/Gal-3-) and were confirmed benign on histology. One case showed loss of CD56 with Gal-3 positivity (CD56-/Gal-3+) and was histopathologically diagnosed as follicular thyroid carcinoma.

Bethesda category IV (n=17): Ten cases displayed a benign immunophenotype (CD56+/Gal-3-) and six displayed a malignant immunophenotype (CD56-/Gal-3+), demonstrating concordance with histopathology in 16/17 cases (94.1%). One discordant case showing co-expression (CD56+/Gal-3+) was confirmed as FVPTC on resection.

Bethesda category V (n=6): Five cases displayed a malignant immunophenotype (CD56-/Gal-3+) and were confirmed malignant on histology. One case showing co-expression (CD56+/Gal-3+) was histopathologically confirmed as a hypercellular colloid nodule. The second benign colloid nodule was in Bethesda category IV.

Diagnostic performance with 95% Confidence Intervals (CI): The diagnostic metrics for CD56 loss, Gal-3 positivity, the combined panel, and FNAC alone relative to histopathology are summarised in [Table/Fig-4].

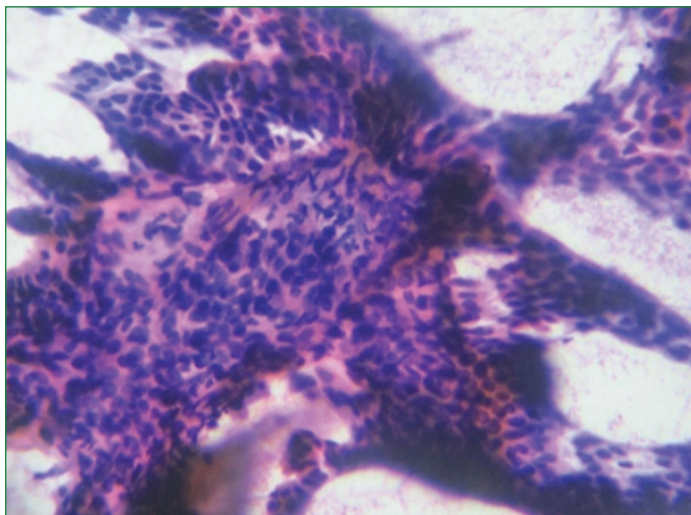
CD56 loss (CD56-): Demonstrated a sensitivity of 91.7% (22/24; 95% CI: 73.0%-99.0%), specificity of 100% (18/18; 95% CI: 81.5%-100%), PPV of 100% (95% CI: 84.6%-100%), NPV of 90.0% (95% CI: 68.3%-98.8%), and diagnostic accuracy of 95.2% (95% CI: 83.8%-99.4%; p<0.001).

Galectin-3 positivity (Gal-3+): Demonstrated a sensitivity of 100% (24/24; 95% CI: 85.8%-100%), specificity of 88.9% (16/18; 95% CI: 65.3%-98.6%), PPV of 92.3% (95% CI: 74.9%-99.1%), NPV of 100% (95% CI: 79.4%-100%), and diagnostic accuracy of 95.2% (95% CI: 83.8%-99.4%; p<0.001).

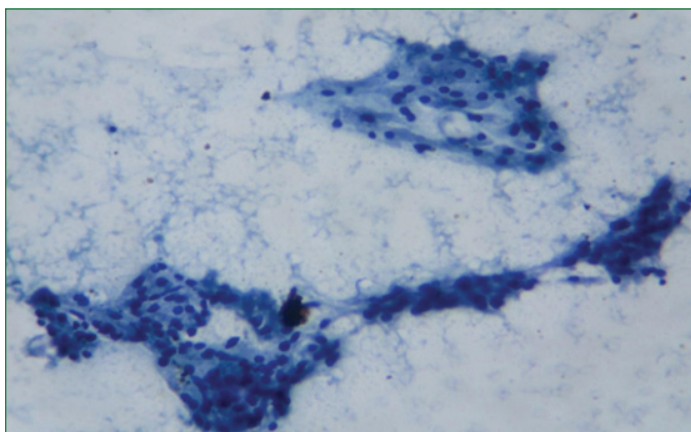
Combined CD56/Gal-3 panel (CD56-/Gal-3+): Under a parallel interpretation rule (where positivity for either marker or Gal-3+ indicates malignancy), the combined panel achieved a sensitivity of 100% (95% CI: 85.8%-100%), specificity of 88.9% (95% CI: 65.3%-98.6%), PPV of 92.3% (95% CI: 74.9%-99.1%), NPV of 100% (95% CI: 79.4%-100%), and overall accuracy of 95.2% (95% CI: 83.8%-99.4%; p<0.001). Because Gal-3 alone detected all 24 malignant cases (100% sensitivity), the calculated sensitivity, specificity, and accuracy for the combined panel were numerically identical to Gal-3 alone. Representative cytological and ICC images are shown in [Table/Fig-5-8].

Comparative performance (FNAC alone vs. FNAC + ICC panel): Conventional FNAC alone demonstrated a sensitivity of 75.0% (18/24; 95% CI: 53.3%-90.2%), specificity of 100% (18/18; 95% CI: 81.5%-100%), PPV of 100% (95% CI: 81.5%-100%), NPV of 75.0% (95% CI: 53.3%-90.2%), and overall diagnostic accuracy of 85.7% (36/42; 95% CI: 71.5%-94.6%). The adjunct application of the dual-marker ICC panel resolved 22 out of 23 cytologically indeterminate

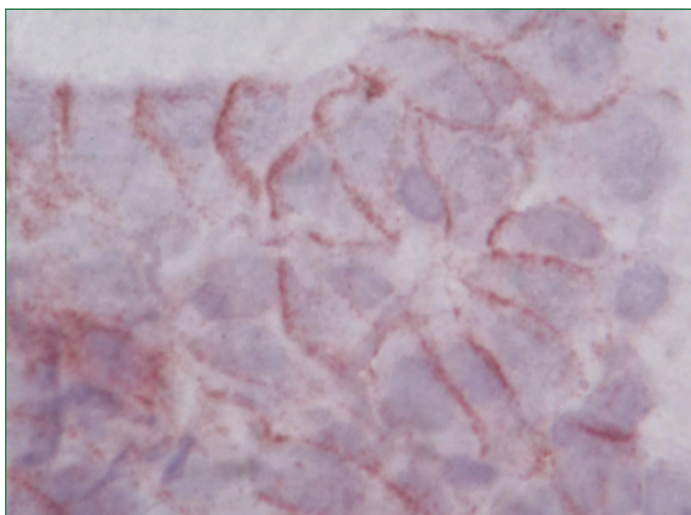
cases (Bethesda III and IV), thereby significantly improving overall diagnostic accuracy compared to FNAC alone (95.2% vs. 85.7%, $p=0.031$ by McNemar's test).



[Table/Fig-5]: Papanicolaou-stained smear showing Papillary Thyroid Carcinoma (PTC) with characteristic nuclear grooves and intranuclear cytoplasmic inclusions (Papanicolaou stain, $\times 400$).



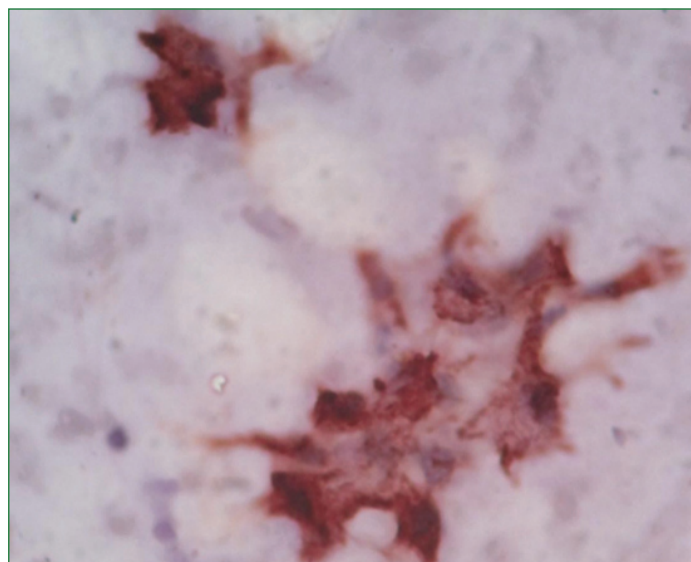
[Table/Fig-6]: Papanicolaou-stained smear showing a follicular-patterned lesion diagnosed cytologically as follicular neoplasm (Papanicolaou stain, $\times 400$).



[Table/Fig-7]: Diffuse membranous CD56 positivity in benign follicular cells (immunocytochemistry, DAB chromogen, $\times 400$).

Cross-tabulations (2×2 contingency matrices) evaluating the individual diagnostic performance of CD56 loss, Gal-3 positivity, and the combined ICC panel against the reference standard (histopathology) were analysed using Fisher's-exact test (given the presence of zero or small expected cell frequencies (<5)). Sensitivity, specificity, PPV, NPV, and overall diagnostic accuracy were reported along with exact 95% binomial CIs. For larger categorical distributions across demographic and cytological categories, Pearson's Chi-

square (χ^2) test was applied where expected cell frequencies met standard requirements (≥ 5).



[Table/Fig-8]: Strong cytoplasmic Gal-3 positivity in Papillary Thyroid Carcinoma (PTC) (immunocytochemistry, DAB chromogen, $\times 400$).

DISCUSSION

The primary diagnostic objective in the cytological assessment of solitary thyroid nodules is to differentiate benign hyperplastic or adenomatous lesions from thyroid malignancies preoperatively [1,5]. FNAC remains the primary initial diagnostic test for thyroid nodules; however, its overall diagnostic accuracy in routine clinical practice is hindered by indeterminate cytological categories—specifically Atypia of Undetermined Significance/Follicular Lesion of Undetermined Significance (AUS/FLUS; Bethesda III) and Follicular Neoplasm/Suspicious for a Follicular Neoplasm (FN/SFN; Bethesda IV) [8,9]. In the present cohort, initial evaluation by FNAC alone yielded a sensitivity of 75.0% (95% CI: 53.3%-90.2%), a specificity of 100% (95% CI: 81.5%-100%), and an overall diagnostic accuracy of 85.7% (95% CI: 71.5%-94.6%). The lower sensitivity of conventional cytology alone stems primarily from cytomorphological overlap in follicular-patterned lesions, where capsular or vascular invasion cannot be demonstrated on smear preparations [12,13].

To mitigate these diagnostic limitations, ancillary biomarker panels applied to cytological smears have emerged as a practical diagnostic strategy. In our cohort, Gal-3 alone achieved absolute sensitivity (100%), correctly identifying all 24 malignant lesions. Consequently, the calculated diagnostic performance parameters of the combined panel (CD56-/Gal-3+) were numerically identical to Gal-3 alone (100% sensitivity, 88.9% specificity, 95.2% accuracy). However, the clinical value of incorporating CD56 lies in its complementary biological role rather than a direct numerical boost in sensitivity.

Standalone Gal-3 testing is known to yield occasional false-positive results in benign colloid nodules, hyperplastic goitres, or thyroiditis. In our study, focal Gal-3 expression (Score 1) occurred in two histologically benign colloid nodules. Crucially, both of these Gal-3-positive benign cases retained strong, diffuse CD56 expression (CD56+). Because CD56 demonstrated 100% specificity across all benign lesions (18/18), its retained membranous expression provided an essential diagnostic “rule-out” signal. Evaluating CD56 alongside Gal-3 prevents diagnostic over-reliance on a single upregulated oncogenic marker, providing cytopathologists with a dual immunophenotypic cross-check (CD56+/Gal-3- for benignity vs. CD56-/Gal-3+ for malignancy) to confirm benign histology when Gal-3 shows equivocal reactivity. CD56, a neural cell adhesion

molecule, is constitutively expressed on normal thyroid follicular cell membranes, where it maintains cell-to-cell adhesion [15,19]. Its downregulation during malignant transformation- particularly in classic PTC and its follicular variants- serves as a reliable negative marker for malignancy [19]. These findings align with the systematic review and meta-analysis by Pyo JS and Kang G (2022), which established that loss of CD56 expression achieves high pooled diagnostic metrics across thyroid neoplasms [21].

Conversely, Gal-3- a 31-kDa β -galactoside-binding lectin implicated in neoplastic transformation, anti-apoptosis, and tumour cell motility- demonstrated a sensitivity of 100% (95% CI: 85.8%-100%), a specificity of 88.9% (95% CI: 65.3%-98.6%), and a diagnostic accuracy of 95.2% (95% CI: 83.8%-99.4%, $p<0.001$). Recent systematic reviews confirm that while Gal-3 is highly sensitive for papillary carcinoma, its standalone diagnostic specificity is occasionally compromised by false-positive expression in benign hyperplastic goitres, lymphocytic thyroiditis, and adenomas [20]. In the present cohort, focal Gal-3 positivity (Score 1) was observed in two histologically benign colloid nodules, reinforcing the clinical caveat that Gal-3 should not be interpreted in isolation [18,20].

When combined into a dual-marker ICC profile (CD56/GD3⁺ for malignancy vs. for benignity), the panel demonstrated a sensitivity of 100% (95% CI: 85.8%-100%), a specificity of 88.9% (95% CI: 65.3%-98.6%), a PPV of 92.3% (95% CI: 74.9%-99.1%), a NPV of 100% (95% CI: 79.4%-100%), and an overall diagnostic accuracy of 95.2% (95% CI: 83.8%-99.4%). Direct comparative analysis revealed that adjunct testing with the CD56/Gal-3 panel significantly improved overall diagnostic accuracy compared to FNAC alone (95.2% vs. 85.7%, $p=0.031$). Crucially, in cytologically indeterminate categories (Bethesda III and IV), the combined panel successfully reclassified 22 out of 23 cases (95.7%) in complete concordance with final histopathology. These empirical results support contemporary practice guidelines recommending dual complementary biomarker panels-pairing an oncogenic up-regulated marker with a physiological down-regulated marker- to optimise risk stratification in thyroid cytopathology [22-24]. This study possesses distinct technical and clinical advantages. First, immunocytochemistry was performed directly on destained Papanicolaou-stained smears. This approach eliminates the requirement for dedicated cell-block preparation or additional needle passes, effectively preserving sparse cytological material while reducing laboratory processing overhead and costs.

From a management standpoint, applying this dual-marker panel directly to routine smears offers a practical, high-yield solution for preoperative triage. This approach is particularly advantageous in resource-constrained health systems where high-throughput molecular testing- such as next- generation sequencing or gene expression profiling- is financially or logistically inaccessible. By improving diagnostic stratification in Bethesda III and IV indeterminate lesions before surgery, this panel has the potential to reduce unnecessary diagnostic hemithyroidectomies, thereby minimising surgical morbidity and optimising clinical resource utilisation.

Limitation(s)

Several methodological limitations of the present study warrant consideration. Most notably, patient inclusion was restricted exclusively to individuals who underwent surgical resection following FNAC, introducing potential verification (work-up) bias. Because cytologically benign lesions are routinely managed conservatively without operative intervention, this surgically selected cohort inherently features a higher baseline malignancy rate than unselected clinical populations. Consequently, while the NPV in general practice may be higher than observed here, the PPV

should be interpreted within the context of a surgically enriched population. Furthermore, the single-centre design and modest sample size limit statistical precision, as reflected by the wider CIs. Future prospective, multicentre validation studies incorporating non surgical cohorts with long-term sonographic follow-up are required to confirm these diagnostic performance parameters in broader clinical settings.

CONCLUSION(S)

In conclusion, direct smear-based immunocytochemistry using a dual-marker panel provides an accessible, cost-effective, and reliable ancillary tool for triaging cytologically indeterminate thyroid nodules. By utilising archival Pap-stained material, this method enhances diagnostic accuracy preoperatively without requiring repeat biopsies or expensive molecular testing, ultimately aiding in safer, more tailored surgical decision-making.

REFERENCES

- [1] Frates MC, Benson CB, Charboneau JW, Cibas ES, Clark OH, Coleman BG, et al. Management of thyroid nodules detected at US: Society of Radiologists in Ultrasound consensus conference statement. *Radiology*. 2005;237(3):794-800.
- [2] Cibas ES, Ali SZ. The Bethesda System for Reporting Thyroid Cytopathology. *Am J Clin Pathol*. 2009;132(4):658-65.
- [3] Ali SZ. Thyroid cytopathology: Bethesda and beyond. *Acta Cytol*. 2011;55(1):4-12.
- [4] Tai JD, Yang JL, Wu SC, Wang BW, Chang CJ. Risk factors for malignancy in patients with solitary thyroid nodules. *J Cancer Res Ther*. 2012;8(3):379-83.
- [5] Fadda G, Rossi ED, Raffaelli M, Pontecorvi A, Sarno MA, Lombardi CP, et al. Follicular thyroid neoplasms can be classified as low- and high-risk according to HBME-1 and Galectin-3 expression. *Eur J Endocrinol*. 2011;165(3):447-53.
- [6] Strickland KC, Howitt BE, Marqusee E, Alexander EK, Cibas ES, Krane JF, et al. Impact of noninvasive follicular variant of papillary thyroid carcinoma. *Thyroid*. 2015;25(9):987-92.
- [7] Bongiovanni M, Spitale A, Faquin WC, Mazzucchelli L, Baloch ZW. The Bethesda System for Reporting Thyroid Cytopathology: a meta-analysis. *Acta Cytol*. 2012;56(4):333-39.
- [8] Cibas ES, Ali SZ, editors. The Bethesda System for Reporting Thyroid Cytopathology: Definitions, Criteria, and Explanatory Notes. 2nd ed. Cham: Springer; 2017.
- [9] Mokhtari M, Eftekhari M, Tahririan R. Absent CD56 expression in papillary thyroid carcinoma: A finding of potential diagnostic value in problematic cases of thyroid pathology. *Journal of Research in Medical Sciences*. 2013;18(12):1046-50.
- [10] Prasad PA, Raju K. Diagnostic utility of CK19 and galectin-3 in differentiating papillary thyroid carcinoma from nonneoplastic lesions of thyroid. *J Cancer Res Ther*. 2022;18(3):644-49. Doi: 10.4103/jcrt.jcrt_563_21
- [11] Lloyd RV, Osamura RY, Klöppel G, Rosai J, editors. WHO Classification of Tumours of Endocrine Organs. 4th ed. Lyon: International Agency for Research on Cancer; 2017.
- [12] Rossi ED, Baloch ZW. Updates in thyroid cytopathology and Bethesda reporting. *Diagn Cytopathol*. 2023;51(8):e1-e10.
- [13] Valderrabano P, McIver B. Evaluation and management of indeterminate thyroid nodules. *Cancer Control*. 2017;24(5):1073274817729231.
- [14] Haugen BR, Alexander EK, Bible KC, Doherty GM, Mandel SJ, Nikiforov YE, et al. ATA management guidelines for thyroid nodules and differentiated thyroid cancer. *Thyroid*. 2016;26(1):1-133.
- [15] Trimboli P, Garelli F, Romanelli F, Crescenzi A, Giovannella L. Role of immunocytochemistry in indeterminate thyroid nodules. *Cancers (Basel)*. 2023;15(16):4120. Doi: 10.3390/cancers15164120.
- [16] Rossi ED, Pantanowitz L. Ancillary testing in thyroid FNAC: current concepts and future directions. *Cancer Cytopathol*. 2024;132(2):89-101. Doi: 10.1002/cncy.22781.
- [17] Ribeiro M, Canberk S, Bongiovanni M. The role of cytology, histology and molecular pathology in the diagnostic process of thyroid nodules. *Cancers (Basel)*. 2026;18(11):1814. Doi: 10.3390/cancers18111814.
- [18] Fu G, Polyakova O, Chazen RS, Freeman JL, Witterick IJ. Diagnostic value of Galectin-3 in distinguishing invasive encapsulated carcinoma from NIFTP. *Cancers (Basel)*. 2021;13(12):2988.
- [19] Trimboli P, Castellana M, Virili C, Dockery F, Giovannella L. Diagnostic performance of Galectin-3 in thyroid lesions: updated systematic review. *Endocr Pathol*. 2022;33(4):520-29. Doi: 10.1007/s12022-022-09727-x.
- [20] Muthusamy S, Azhar Sha S, Abdullah Suhaimi SN, Kassim N, Mahasin M, Mohd Saleh MF, et al. CD56 expression in benign and malignant thyroid lesions. *Malays J Pathol*. 2018;40(2):111-19.
- [21] Pyo JS, Kang G. Diagnostic value of CD56 loss in papillary thyroid carcinoma: meta-analysis. *Pathol Res Pract*. 2022;240:154184.
- [22] El Demellawy D, Nasr AL, Babay S. Diagnostic utility of CD56 immunohistochemistry in papillary carcinoma of the thyroid. *Pathol Res Pract*. 2009;205(5):303-09.

[23]

Bizzarro T, Martini M, Marrocco C, D'Amato D, Traini E, Lombardi CP, et al. The role of CD56 in thyroid fine needle aspiration cytology. PLoS One. 2015;10(7):e0132939.

[24]

Hirokawa M, Suzuki A. Immunocytochemistry in cytology: myth or reality: unraveling the myth- immunocytochemistry applications in thyroid lesions. Acta Cytol. 2025;69(1):7-15. Doi: 10.1159/000540366.

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