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Pre-operative diagnosis of suspicious thyroid nodules – an integrated multidisciplinary approach

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ABSTRACT

Introduction and aim. Thyroid nodules are common, though few are malignant. In lower-middle-income countries such as Sri Lanka, limited diagnostic resources make accurate preoperative assessment essential to avoid unnecessary surgery. While the Thyroid Imaging reporting and Data System (TIRADS) and Bethesda systems are widely used, both have limitations, particularly in differentiating follicular lesions. This study aimed to evaluate the potential value of immunocytochemistry (ICC) as an adjunct to preoperative diagnosis in suspicious thyroid nodules.

Material and methods. A prospective observational diagnostic accuracy study was conducted on 106 patients with TIRADS ≥3 nodules. Fine-needle aspiration cytology was classified using the Bethesda system, and cell blocks from 34 cases underwent ICC with HBME-1, CK-19, and CD-56. Final diagnoses were established using histopathology where available or a composite reference standard based on stable imaging follow-up over two years. Diagnostic performance was assessed using logistic regression, ROC curves, and confusion matrices.

Results. Bethesda showed sensitivity of 80.8% and specificity of 60.6% (AUC=0.73), while TIRADS showed sensitivity of 89.0% and specificity of 30.3% (AUC=0.65). Among the ICC-tested cases, specificity was 100%;

sensitivities were 94.1% for HBME-1, 88.2% for CD-56, and 41.2% for CK-19. The HBME-1⁺/CD-56⁻ pattern was strongly associated with malignancy. The combined Bethesda+ICC model demonstrated the best predictive performance (lowest AIC). Papillary carcinoma was the most frequent malignancy.

Conclusion. In this cohort, ICC using HBME-1, CK-19, and CD-56 showed potential as an adjunct to the Bethesda classification for the preoperative assessment of thyroid nodules. These findings suggest a possible role for ICC in selected cases, although further studies with larger histologically confirmed cohorts are needed.
Keywords. Bethesda, immunocytochemistry, thyroid cancer, TIRADS

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Introduction

Thyroid nodules are a common clinical finding, although only a small proportion are malignant. Accurate preoperative diagnosis is therefore essential to ensure appropriate patient management and to avoid unnecessary surgical intervention.[1] With the global incidence of thyroid carcinoma continuing to rise, reliable methods for distinguishing benign from malignant thyroid nodules remain an important clinical priority.[2]

Ultrasonography (USS) and fine-needle aspiration cytology (FNAC) are the principal preoperative diagnostic tools used in the evaluation of thyroid nodules. USS findings are commonly reported using the Thyroid Imaging Reporting and Data System (TIRADS), while FNAC results are classified according to the Bethesda System for Reporting Thyroid Cytopathology.[3] These approaches play a central role in risk stratification and surgical decision-making. However, both modalities have recognized limitations, particularly in the evaluation of follicular-patterned lesions, as they provide limited information regarding tissue architecture. As a result, distinguishing benign follicular adenomas from malignant follicular neoplasms may remain challenging, leading to diagnostic uncertainty and, in some cases, unnecessary surgical procedures.

This diagnostic challenge is particularly relevant in lower-middle-income countries such as Sri Lanka, where healthcare resources are limited and avoiding unnecessary surgery is an important clinical and economic consideration. Although histopathological examination remains the gold standard for the diagnosis of thyroid tumors, it requires surgical excision and therefore cannot be used as a preoperative diagnostic tool. Furthermore, both cytological and histological assessment of thyroid lesions may be complicated by the gland's predominantly follicular architecture and nodular growth patterns.[4]

To improve preoperative diagnostic accuracy, several immunomarkers have been investigated as adjuncts to conventional cytological assessment. While these markers are traditionally applied to surgically resected specimens, they can also be evaluated on FNAC-derived cell block preparations using immunocytochemistry (ICC). This approach utilizes material obtained during routine FNAC and may provide additional diagnostic information without requiring more invasive procedures. Therefore, ICC performed on FNAC-derived cell blocks has the potential to serve as a practical adjunct to existing diagnostic methods, particularly in resource-limited settings. This study aimed to assess the value of selected ICC markers in improving the preoperative diagnosis of suspicious thyroid nodules.

Aim

Accordingly, the present study aimed to assess the utility of immunocytochemistry as an adjunctive technique to improve the accuracy of preoperative diagnosis in suspicious thyroid nodules. This study further explored the feasibility and potential diagnostic contribution of immunocytochemistry performed on FNAC-derived cell blocks alongside conventional TIRADS and Bethesda assessment in a Sri Lankan resource-limited setting, where access to advanced molecular diagnostic techniques is limited.

Material and methods

A prospective observational diagnostic accuracy study was carried out at the Departments of Radiology and Pathology of Colombo South Teaching Hospital (CSTH), Sri Lanka, and the Department of Pathology, Faculty of Medical Sciences (FMS), University of Sri Jayewardenepura (USJP). A total of 106 patients presenting with thyroid nodules radiologically suspected to be malignant (TIRADS category 3 or higher) on ultrasonography were recruited for the study. Patients with incomplete or inadequate radiological data were excluded. Ethical approval for the study was obtained from the Ethics Review Committee of the Faculty of Medical Sciences, USJP (Ref. No. 65/2019). Written informed consent was obtained from all study participants.

Ultrasonography (USS) examinations were performed and reported by a consultant radiologist according to the TIRADS classification. Ultrasound-guided fine-needle aspirations (FNAs) were subsequently obtained from the radiologically suspected malignant nodules by the same radiologist. Direct smears were prepared from the aspirated material, while the residual needle rinse was used for cell block preparation. Cell blocks were processed using the alcohol-acid-formalin (AAF) method. Both the direct smears and cell block sections were stained with hematoxylin and eosin (H&E). The stained preparations were independently examined by two consultant pathologists and categorized according to the Bethesda System for Reporting Thyroid Cytopathology.

ICC staining was performed on adequately cellular FNAC-derived cell block sections using a manual streptavidin–biotin peroxidase method. Heat-induced epitope retrieval was carried out in citrate buffer (pH 6.0) using a pressure cooker. The immunocytochemical panel consisted of HBME-1 (Invitrogen; diluted 1:24), CK-19 (Dako; ready-to-use), and CD56 (Dako; ready-to-use). Following antigen retrieval, the sections were incubated with the respective primary antibodies for one hour. A secondary antibody and 3,3′-diaminobenzidine (DAB) chromogen were subsequently applied, and the slides were counterstained with hematoxylin. Appropriate positive and negative controls were included in each staining batch. The stained slides were independently evaluated by two consultant pathologists who were blinded to the cytological diagnosis.

For interpretation, cytoplasmic and/or membranous staining patterns were assessed according to the specific marker characteristics. HBME-1 and CK-19 were considered positive when moderate to strong membranous or cytoplasmic staining was observed in more than 10% of tumor cells, whereas CD56 expression was interpreted as positive when diffuse membranous staining was detected in more than 10% of follicular cells.

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Loss of CD56 expression was considered supportive of malignancy. If any discrepancies were observed between the two pathologists, a joint slide review followed by a consensus discussion was undertaken to resolve the differences and ensure consistency and accuracy in the final interpretations.

All patients included in the study were prospectively followed over a period of two years to determine the final outcome of their thyroid nodules as benign or malignant. For those who underwent surgical excision during the follow-up period, the definitive diagnosis was established through histopathological examination of the resected specimen. Among patients who did not undergo surgery, nodules that were classified as TIRADS 3 or 4 on ultrasonography or reported as non-diagnostic on initial FNAC were re-evaluated with repeat FNAC. In addition, serial ultrasonographic assessments were performed at six-month intervals to monitor changes in nodule size, morphology, or the appearance of suspicious features. If, during the follow-up period, any ultrasound scan revealed a nodule categorized as TIRADS 3 or above, a repeat FNAC was carried out.

The reference standard for malignancy was based on a composite outcome. Nodules reported as Bethesda category II on cytological evaluation were considered benign and managed with surveillance. Surgical intervention was undertaken when indicated based on comprehensive clinical, radiological, and cytological assessment (e.g., suspicion of malignancy, tracheal compression, significant cosmetic concerns, or poor compliance with follow-up). In patients who underwent surgery, the final diagnosis was determined by histopathological examination.

In the absence of surgical indications, patients were followed for two years. Nodules that remained stable in size and imaging characteristics and did not require surgery during follow-up were classified as benign for this study.

Clinical management was undertaken through standard multidisciplinary evaluation involving surgeons, radiologists, and the treating team. The estimated risk of malignancy was communicated to patients, and decisions regarding surgical intervention were made following shared clinical discussions integrating imaging, cytological, and clinical findings.

Data analysis was performed using the R language (version 4.3.1). Descriptive statistics were presented as frequencies and proportions. Diagnostic accuracy calculations were based on a composite reference standard consisting of histopathological diagnosis in surgically treated patients and two-year clinical and radiological follow-up in patients who did not undergo surgery. Fisher's exact test was employed to evaluate associations between categorical diagnostic variables. Logistic regression analysis was conducted to quantify the strength and direction of associations among relevant variables. The diagnostic performance of the tests was further assessed using the receiver operating characteristic (ROC) curve analysis. Sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) were calculated based on the confusion matrix. A p-value of less than 0.05 was considered statistically significant. Logistic regression was performed to evaluate the predictive performance of cytological, radiological, and immunohistochemical classification systems for malignancy. Six predefined models were assessed: (1) ICC×Bethesda, (2) ICC×TI-RADS, (3) ICC alone, (4) Bethesda×TI-RADS, (5) Bethesda alone, and (6) TI-RADS alone. Model performance was compared using the

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Akaike Information Criterion (AIC), with lower values indicating better relative model fit. AIC was used for comparative evaluation within the dataset and does not imply absolute predictive superiority. Models were constructed based on clinical relevance without automated variable selection.

Results

The majority of the participants in the study were females (n=84, 79.2%). The age of the enrolled participants ranged from 15 to 76 years, with a mean and standard deviation of 47.20 ± 12.90, respectively. All 106 radiologically malignancy-suspected patients were subjected to FNAC. Of them, 78 (73.58%) were diagnosed as cytologically benign, while 28 (26.41%) were diagnosed as malignant or suspicious of malignant. At the two-year follow-up, 24 of the 78 cytologically benign patients had undergone surgery. Of those 24 patients, 15 patients were histologically confirmed to have malignant nodules, while 9 had benign nodules. Of the 28 cytologically malignant or malignancy-suspected patients, only 21 had undergone surgery. Of those 21 patients, 18 were histologically confirmed to have malignant tumors, while 3 patients were confirmed to have benign nodules.

As only patients with radiologically suspected malignancies were enrolled in the study, all patients were classified in TIRADS 3 or above categories. The majority (n=80, 75.4%) of the nodules belonged to the TIRADS 4 category (TIRADS 4a: n=54, TIRADS 4b: n=8, TIRADS 4c: n=18). Furthermore, 21 nodules in the TIRADS 3 cohort and 5 in the TIRADS 5 cohort were also reported.

Although all 106 enrolled patients had radiologically malignant nodules, only 28 (26.4%) had cytologically diagnosed with suspicion of malignant (Bethesda 5: n=20, 18.9%) or malignant (Bethesda 6: n=8, 7.5%) nodules. The majority (n=43, 40.6%) of the nodules belonged to the benign category (Bethesda 2). Furthermore, 15 Bethesda 3 nodules, 14 Bethesda 1 nodules, and 6 Bethesda 4 nodules were also reported (Fig 3). Of the 20 cytologically malignant-suspected nodules, the majority (n=10) belonged to the TIRADS 4c category. The other ten patients were classified into TIRADS 4a (n=6), TIRADS 3 (n=2), TIRADS 4b (n=1), and TIRADS 5 (n=1) categories. Of the 8 cytologically malignant patients, 6 were classified in TIRADS 4c, and the other 2 cases were distributed in one per each of TIRADS 4b and TIRADS 5 categories (Table 1).

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Table 1. Association of TIRADS classification and FNAC results*

TIRADS category vs direct cytology cross tabulation								
		Direct cytology – Bethesda category						**Total**
		1	2	3	4	5	6	
TIRADS Category	3	1	14	3	1	2	0	21
	4a	11	26	9	2	6	0	54
	4b	1	2	1	2	1	1	8

	4c	1	0	1	0	10	6	18
	5	0	1	1	1	1	1	5
Total		14	43	15	6	20	8	106

* Non-diagnostic or unsatisfactory – Bethesda 1, benign – Bethesda 2, atypia of undetermined significance or follicular lesion of undetermined significance – Bethesda 3, follicular neoplasm or suspicious of follicular neoplasm – Bethesda 4, suspicious for malignancy – Bethesda 5, malignant – Bethesda 6

Among the forty-five (n=45) patients who had undergone surgery, thirty-three (n=33) were histologically confirmed to have malignant tumors, while the remaining twelve (n=12) were confirmed to have benign nodules. Of the 33 confirmed malignant cases, only 18 were both radiologically and cytologically malignancy suspected/ malignant (papillary thyroid carcinoma: n=16, papillary microcarcinoma: n=02), while the other 15 cases were cytologically non-malignant (papillary thyroid carcinoma: n=10, follicular variant of papillary thyroid carcinoma: n=03, cystic papillary thyroid carcinoma: n=01 and papillary microcarcinoma: n=01). However, radiologically and cytologically malignancy suspected three cases (Chronic thyroiditis: n=2, hyperplastic nodule: n=1) were histologically confirmed to have benign nodules. Nine patients were radiologically, cytologically, and histologically confirmed to have benign nodules (chronic thyroiditis: n=3; multinodular goiter: n 3; hyperplastic nodule: n 2; and colloid cyst: n=1).

Among the forty-five patients who underwent surgery, papillary thyroid carcinoma was identified as the most common type (n=26) of malignant tumor (TIRADS 4a=10, TIRADS 4c=9, TIRADS 4b=3, TIRADS 3 =3, TIRADS 5=1). In addition, 3 papillary microcarcinoma cases (TIRADS 3: n=1, TIRADS 4c: n=1, TIRADS 5: n=1), 3 follicular variants of papillary carcinoma (TIRADS 3: n=1, TIRADS 4a: n=1, TIRADS 4b: n=1), and one cystic papillary carcinoma case (TIRADS 5=1) were reported.

Of the twelve (n=12) patients who were histologically confirmed to have benign nodules, 5 had chronic thyroiditis (TIRADS 3: n=2, TIRADS 4c:n=2, TIRADS 4a: n=1), while three had hyperplastic nodules (TIRADS 4a =3), two had multinodular goiter with thyroiditis (TIRADS 4a n=2), and one had a colloid cyst (TIRADS 3).

At the two-year follow-up, seventy-three (n=73) patients had clinically benign outcomes (TIRADS 3: n=15, TIRADS 4a: n=38, TIRADs 4b: n=5, TIRADS 4c: n=18, TIRADS 5: n=5).

Of the 26 patients with confirmed papillary thyroid carcinoma (PTC), sixteen were cytologically malignancy suspected (Bethesda 5: n=10) or malignant (Bethesda 6: n=6), while 6 cases were in Bethesda 2, 2 cases in Bethesda 1, and 1 case each in Bethesda 3 and 4 categories. However, the three papillary microcarcinoma cases belonged to the Bethesda 2 (n=1), Bethesda 5 (n=1), and Bethesda 6 (n=1) categories.

All three (n=3) follicular variants of papillary thyroid carcinoma patients were in cytologically nonmalignant categories (Bethesda 2: n=1, Bethesda 3: n=1, Bethesda 4: n=1), while they were radiologically classified under TIRADS 3, 4a, and 4b categories. Furthermore, one (n=1) cystic papillary thyroid carcinoma case was

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reported, and it was classified as Bethesda category 5 and TIRADS category 5.

Standardized residual analysis demonstrated a significant association between TIRADS classifications (≥TIRADS 3) and Bethesda categories, indicating that nodules with higher TIRADS scores were more likely to align with higher Bethesda categories, particularly among radiologically suspected malignant nodules. A statistically significant correlation was observed between TIRADS classification (≥TIRADS 3) and Bethesda categories ($p=0.00009$). Additionally, Fisher's exact test revealed a significant association between TIRADS classification and the benign-malignant status of patients at two-year follow-up ($p=0.046$). Notably, the TIRADS 4c category had a significant contribution to this association ($p=0.047$), with nodules in this category demonstrating a four-fold increased risk of malignancy compared to those classified as TIRADS 3 (Table 2). ROC curve analysis further indicated that the TIRADS system possessed a limited to modest discriminatory ability to differentiate between benign and malignant lesions, with an area under the curve (AUC) of 0.65 (Fig. 1).

Table 2. Logistic regression data analysis – TIRADS classification

TIRADS classification	Odd ratio	95% CI[1]	p
3	–	–	
4a	0.91	0.29, 3.24	0.900
4b	3.20	0.57, 18.9	0.200
4c	4.00	1.06, 16.9	0.047
5	2.13	0.23, 16.9	0.500

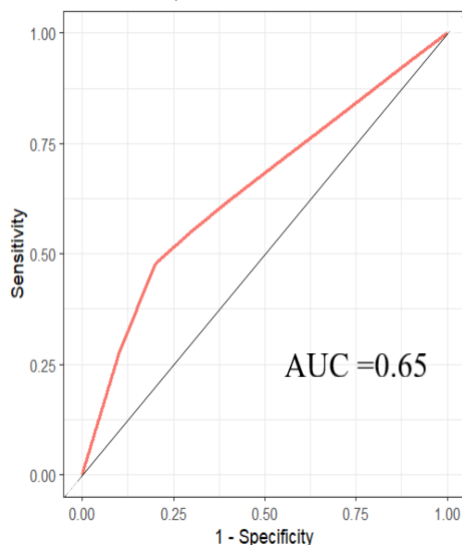

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Fig. 1. ROC curve for the ultrasound scan findings (AUC=0.5: model performs like random guessing, AUC=1.0: model perfectly separates positive and negative cases, AUC > 0.5: model has some ability to predict correctly)

Furthermore, a statistically significant association was identified between the Bethesda classification and the benign-malignant status of patients at the two-year follow-up (p=0.00143). Logistic regression analysis revealed that patients classified as Bethesda category 6 had a significantly increased likelihood of malignancy, with an odds ratio of 13.1 (95% CI: 2.52–102; p=0.004), compared to those in Bethesda category 2. Similarly, individuals in Bethesda category 5 exhibited a fivefold higher risk of malignant outcomes (OR: 5.0; 95% CI: 1.70–18.0; p=0.005) (Table 3). The Bethesda system also demonstrated a moderate discriminatory capacity for distinguishing between benign and malignant lesions, as indicated by an AUC of 0.73 (Fig. 2)

Table 3. Logistic regression data analysis – Bethesda classification

Bethesda classification	Odd ratio	95% confidence interval	p
Bethesda 02	–	–	–
Bethesda 03	0.67	0.09, 4.99	0.600
Bethesda 04	4.38	0.70, 27.9	0.100
Bethesda 05	5.35	1.70, 18.0	0.005
Bethesda 06	13.1	2.52, 102	0.004

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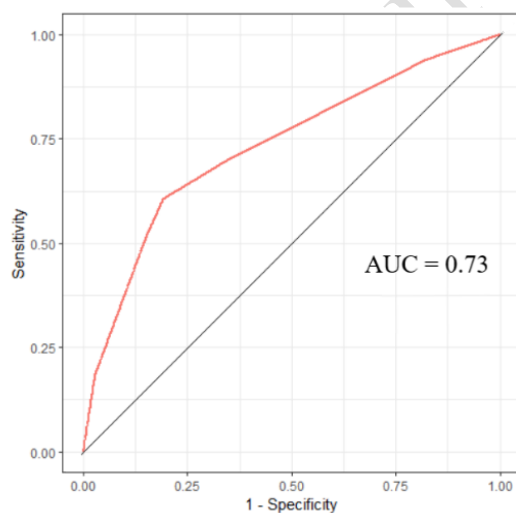

Fig. 2. ROC curve of FNAC findings compared to histology (AUC=0.5: model performs like random guessing., AUC=1.0: model perfectly separates positive and negative cases, AUC > 0.5: model has some ability to predict correctly)

Diagnostic performance was evaluated separately in the histology-verified subgroup and the entire cohort. In the histology-verified subgroup, where histopathology served as the gold standard, TIRADS (≥TR4) demonstrated a sensitivity of 94.3%, specificity of 22.2%, PPV of 77.3%, NPV of 57.1%, and accuracy of 75.0%. In comparison, Bethesda classification showed a sensitivity of 62.9%, specificity of 77.8%, PPV of 88.0%, NPV of 46.7%, and accuracy of 67.3%. In the entire cohort, using a composite reference standard, TIRADS showed a sensitivity of 89.04%, specificity of 30.30%, PPV of 73.86%, NPV of 55.56%, and accuracy of 70.75%, whereas Bethesda demonstrated a sensitivity of 80.82%, specificity of 60.61%, PPV of 87.94%, NPV of 58.82%, and accuracy of 74.53% (Fig. 3).

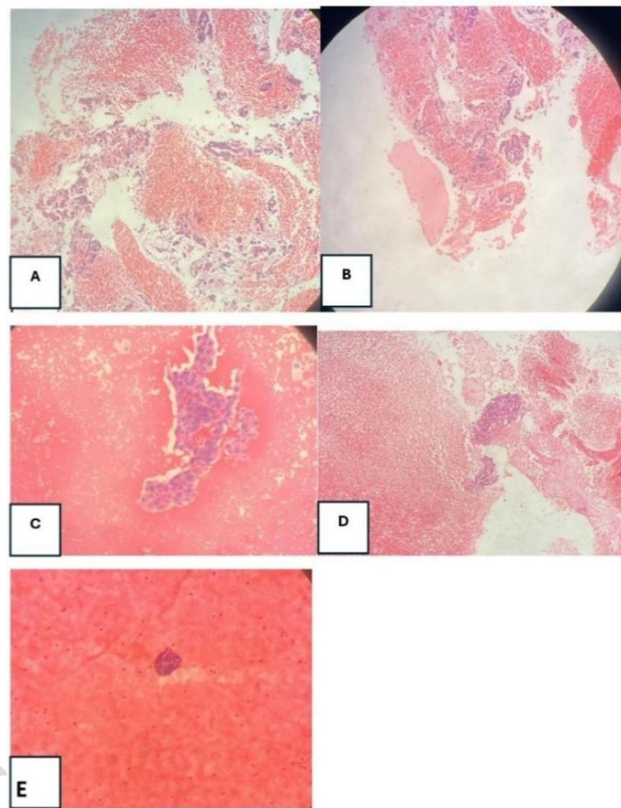

Fig. 3. Microscopic appearance of cell block sections and direct cytology smears, A: Cell block section showing papillary structures characteristic of papillary thyroid carcinoma (H&E stained, ×100 magnification), B: Cell block section showing nuclear pseudoinclusions typical of papillary thyroid carcinoma (H&E stained, ×100 magnification), C: Direct smear of papillary thyroid carcinoma displaying nuclear pseudoinclusions (H&E stained, ×200 magnification), D: Cell block section demonstrating hyperplastic benign nodules of a hyperplastic thyroid nodule (H&E stained, ×200 magnification), E: Direct smear showing benign follicular epithelial cells in a hyperplastic nodule (H&E stained, ×100 magnification)

ICC was performed on 34 FNAC-derived cell blocks with adequate cellularity. Of these, 17 (50.0%) cases were negative for HBME-1, 16 (47.1%) were positive, and one (2.9%) was focally positive. All HBME-1-negative

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cases were negative for CK-19 and positive for CD56. In contrast, among the HBME-1-positive or focally positive cases, the majority demonstrated positive or focally positive CK-19 expression together with loss of CD56 expression (Table 4) (Fig. 4).

Table Błąd! W dokumencie nie ma tekstu o podanym stylu.. Frequency of HBME-1, CK-19 and CD-56 immunomarker

IHC_HBME1			**IHC_CD56**			**Total**
			Negative	**Positive**	**Equivocal**	
Negative	IHC_CK19	Negative		17		17
	Total			17		17
Positive	IHC_CK19	Negative	5		0	5
		Positive	7		0	7
		Focal positive	2		2	4
	Total		14		2	16
Focal Positive	IHC_CK19	Focal positive	1			1
	Total		1			1
Total	IHC_CK19	Negative	5	17	0	22
		Positive	7	0	0	7
		Focal positive	3	0	2	5
	Total		15	17	2	34

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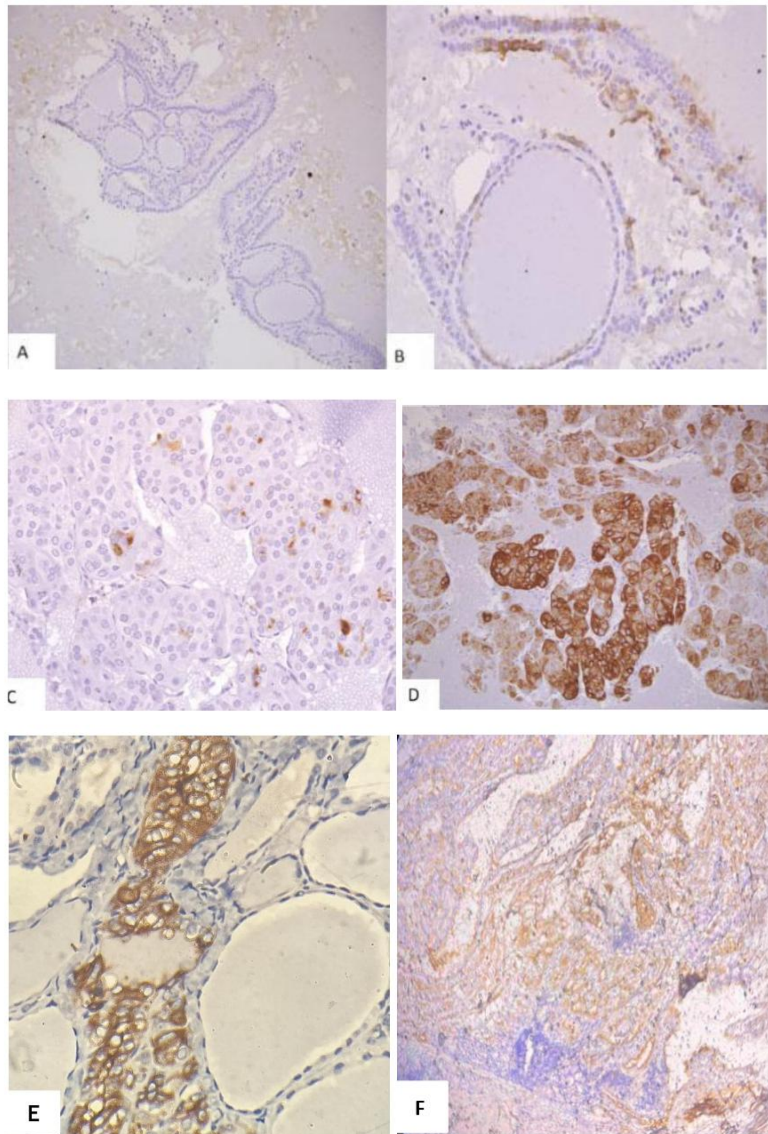

Fig. 4. IHC staining patterns observed in the study, A: Negative staining for HBME-1 (×40 magnification), B: Focal staining for CK-19 (×40 magnification), C: Focal staining for HBME-1 (×200 magnification), D: Positive staining for CK-19 (×200 magnification), E: Positive staining for HBME-1 (×200 magnification), F. Positive staining for CD-56 (×200 magnification)

A statistically significant association was observed between the expression patterns of HBME-1, CK-19, and CD56 and the final benign-malignant status of thyroid nodules at two-year follow-up ($p<0.001$). All three markers demonstrated 100% specificity. Sensitivity was highest for HBME-1 (94.11%), followed by CD56 (88.23%) and CK-19 (41.17%). ROC analysis yielded AUC values of 1.00 for HBME-1, 0.853 for CK-19, and 0.882 for CD56.

Of the 34 cases evaluated by ICC, 24 had histopathological confirmation, while 10 were classified according to the two-year follow-up reference standard. The ten non-operated cases were negative for

HBME-1 and CK-19 and positive for CD56 and demonstrated benign clinical outcomes throughout follow-up.

When ICC findings were compared with the final diagnosis, all nodules negative for HBME-1 and CK-19 but positive for CD56 were classified as benign. Conversely, all nodules demonstrating positive or focally positive HBME-1 staining were classified as malignant. Among the 17 malignant cases, CK-19 was positive in seven (n=7) cases, focally positive in five (n=5) cases, and negative in five (n=5) cases. CD56 expression was absent in 15 cases, while two (n=2) cases demonstrated equivocal staining.

PTC was the most common malignant lesion identified in the ICC subgroup, accounting for 12 of 34 cases (35.3%). All PTC cases demonstrated positive or focally positive HBME-1 staining. CK-19 expression was positive in four cases, focally positive in five cases, and negative in three cases. CD56 expression was absent in 11 of 12 PTC cases, while one case demonstrated equivocal staining. No PTC case demonstrated the combination of HBME-1 negativity and CD56 positivity.

Among the papillary microcarcinoma cases (n=2), one showed positivity for both HBME-1 and CK-19 with loss of CD56 expression, while the other was positive for HBME-1, negative for CK-19, and negative for CD56. The cystic and follicular variants of papillary thyroid carcinoma each demonstrated positive HBME-1 and CK-19 staining with loss of CD56 expression. The multifocal PTC case was positive for HBME-1 and negative for both CK-19 and CD56.

All benign lesions, including chronic thyroiditis (n=4), colloid cyst (n=1), hyperplastic nodule (n=1), and multinodular goiter with thyroiditis (n=1), demonstrated the characteristic staining pattern of HBME-1 negativity, CK-19 negativity, and CD56 positivity.

The predictive performance of six logistic regression models was compared using the Akaike Information Criterion (AIC), with lower values indicating better relative model fit. The combined immunocytochemistry (ICC) and Bethesda classification model demonstrated the lowest AIC value (AIC=4), indicating the best relative fit among the evaluated models. The ICC×TIRADS model showed the second-lowest AIC value (AIC=12), followed by ICC alone (AIC=14), Bethesda×TIRADS (AIC=18), Bethesda alone (AIC=20), and TIRADS alone (AIC=22). Overall, models incorporating ICC showed lower AIC values than models based solely on TIRADS or Bethesda classifications. These findings suggest that the addition of ICC may improve the relative predictive performance of conventional classification systems in identifying malignant thyroid nodules within this study cohort.

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Discussion

The observed female predominance and peak incidence in the fourth to fifth decades are consistent with established epidemiological patterns in thyroid neoplasia.[6–8] These findings support previously reported associations between thyroid neoplasia and biological susceptibility factors, including hormonal influences and X-chromosome inactivation, although the exact mechanisms remain incompletely understood.[9]

The relatively higher false-negative rate observed in the cytology findings highlights recognized limitations of FNAC in routine clinical practice. While false-positive results were infrequent, false-negative findings may result from sampling inadequacy, operator-dependent factors, and overlapping cytomorphological features between benign and malignant lesions.[10] In particular, atypical cyst lining cells, granular cytoplasm, cytoplasmic processes, small micronodules, and subtle nuclear features may contribute to diagnostic uncertainty and misclassification.[11,12] These findings reinforce the well-recognized challenges associated with the diagnosis of follicular-patterned lesions using cytology alone.

Misclassification of follicular variants of papillary thyroid carcinoma further illustrates the inherent limitations of cytology. Subtle nuclear alterations, such as nuclear enlargement, membrane irregularities, and chromatin clearing, may overlap with benign reactive changes when present in a limited number of cells, thereby reducing diagnostic specificity.[12] This reinforces the recognized difficulty in distinguishing follicular-patterned lesions using cytology alone.

The significant association between higher TIRADS categories (4c and 5) and malignancy supports the clinical utility of ultrasound-based risk stratification. However, the magnitude of risk observed in the present study was lower than that reported in previous studies, including an Indian study demonstrating higher risk estimates across TIRADS categories.[13] Despite these differences, the progressive increase in malignancy risk with higher TIRADS categories supports its role as a stratification tool rather than a standalone diagnostic method.

Comparison of TIRADS and Bethesda classification demonstrated complementary diagnostic strengths. In the histology-verified subgroup, TIRADS showed higher sensitivity, supporting its role in reducing missed malignancies, whereas the Bethesda classification demonstrated higher specificity and positive predictive value. A similar pattern was observed in the overall cohort using the composite reference standard. These findings are broadly comparable to those reported by George et al., although differences in specificity and overall diagnostic performance may reflect variations in case selection, inclusion of indeterminate nodules, and the use of a composite reference standard.[13] The comparatively lower diagnostic accuracy relative to larger cohorts[14] further highlights the influence of case mix and methodological differences. Collectively, these findings support the complementary use of radiological and cytological assessment in the evaluation of thyroid nodules.

The observed differences in diagnostic performance between the histology-verified subgroup and the overall cohort may be explained by verification bias and the use of a composite reference standard. Histopathology-based evaluation provides a definitive diagnosis but is limited to surgically treated patients, who are more likely to have a higher risk of malignancy. In contrast, inclusion of non-operated cases in the overall cohort may introduce potential misclassification, particularly among patients managed conservatively. Therefore, these findings should be interpreted in light of the applied reference standards and the characteristics of the study population.

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The present study demonstrates the added value of immunocytochemistry in improving diagnostic precision, particularly in indeterminate cases. The high specificity observed for HBME-1, CK-19, and CD-56 supports their utility as adjunctive markers.[15,16] The superior performance of HBME-1 is consistent with its established association with papillary thyroid carcinoma[17,18], while lower sensitivities reported in other studies may reflect differences in tumour spectrum.[19,20] Although CK-19 demonstrated lower sensitivity, its high specificity supports its role in excluding malignancy when negative.[21] The absence of CD-56 expression in malignant nodules further reinforces its inverse association with malignancy.[22–24]

The marker expression patterns observed in this study are consistent with the concept of panel-based interpretation. The combination of HBME-1 positivity, CK-19 positivity, and loss of CD-56 expression was associated with malignant lesions, whereas benign lesions typically demonstrated the opposite staining profile. Similar observations have been reported previously, supporting the potential value of combined marker assessment.[25] This approach may be particularly relevant in resource-limited settings where access to advanced molecular diagnostic techniques is limited.[26–28] In addition, subtype-specific expression patterns observed in papillary thyroid carcinoma and its variants were broadly consistent with previous reports.[29,30] However, given the relatively small number of cases evaluated by immunocytochemistry, these findings should be regarded as preliminary and require validation in larger cohorts before firm conclusions can be drawn.

Although markers such as galectin-3, BRAF VE1, and TROP-2 have demonstrated diagnostic value in thyroid cytology, they were not included in the present study because they were not part of the routinely applied panel during the study period. Future studies incorporating broader immunocytochemical and molecular panels may provide additional diagnostic information.

In the present study, the AUC for TIRADS was 0.65, indicating limited to modest discriminatory ability. Although this suggests some capacity to differentiate between benign and malignant lesions, such performance does not reflect strong diagnostic accuracy. Therefore, TIRADS classification alone may be insufficient to guide definitive clinical decision-making, and its findings should be interpreted in conjunction with cytological, immunocytochemical, and clinical parameters.

Comparative AIC analysis suggested that the combined immunocytochemistry and Bethesda classification model provided the most favorable relative fit among the evaluated models. Models incorporating immunocytochemistry generally demonstrated lower AIC values than models based solely on TIRADS or Bethesda classifications. However, these findings should be interpreted cautiously. The relatively small number of cases available for immunocytochemistry increases the risk of overfitting and limits the generalizability of model-based inferences. Consequently, the observed differences in AIC values should be regarded as exploratory indicators of relative model fit rather than evidence of superior predictive performance.

Several limitations should be considered when interpreting the findings of this study. Histopathological

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confirmation was available only for patients who underwent surgical excision, creating the potential for verification bias. Patients managed conservatively were classified according to cytological findings and imaging stability during two years of follow-up, which reflects routine clinical practice but may not completely exclude indolent malignancies. Furthermore, the relatively small number of malignant outcomes and immunocytochemistry-tested cases may have increased the risk of model overfitting and reduced the precision of multivariable estimates. The wide confidence intervals observed for several predictors further suggest uncertainty in some effect estimates. Therefore, the multivariable and model-comparison findings should be interpreted as exploratory and require confirmation in larger, independent cohorts.

Conclusion

This study demonstrates that while TIRADS and the Bethesda system are valuable tools in the preoperative evaluation of thyroid nodules, each has inherent limitations, particularly in the assessment of follicular-patterned lesions. The findings suggest that immunocytochemistry using a panel of HBME-1, CK-19, and CD-56 may provide useful additional diagnostic information when used as an adjunct to conventional radiological and cytological assessment. The combined use of immunocytochemistry with Bethesda classification showed improved predictive performance within the study cohort and may help reduce diagnostic uncertainty in selected cases. These findings indicate a potential role for immunocytochemistry in supporting preoperative decision-making, particularly in resource-limited settings where access to advanced molecular diagnostics is restricted. However, given the relatively small number of cases evaluated by immunocytochemistry, further validation in larger, independent cohorts is required before its routine clinical application can be recommended.

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Declarations

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Author contributions

Conceptualization, H.K, B.S., N.F., A.P, B.G. and A.S.; Methodology, H.K., B.S., A.P. and B.G.; Software, H.K.; Validation, H.K., B.S., N.F, A.P., and B.G.; Formal Analysis, H.K and B.S.; Investigation, H.K., B.S., N.F, A.P., and B.G.; Resources, B.S. and N.F..; Data Curation, H.K, B.S., N.F., A.P, B.G. and A.S.; Writing – Original Draft Preparation, H.K.; Writing – Review & Editing, H.K, B.S., N.F., A.P, B.G. and A.S.; Visualization, H.K.; Supervision, B.S., N.F., A.P, B.G. and A.S.; Project Administration, B.S., N.F., A.P, B.G. and A.S.; Funding Acquisition, B.S and N.F.

Conflicts of interest

The authors have no financial or proprietary interest in any material discussed in this article.

Data availability

Data supporting the findings of this study are available from the corresponding author upon reasonable request.

Ethics approval

Ethical approval for the study was obtained from the ethics review committee of the Faculty of Medical Sciences, University of Sri Jayewardenepura (65/19) and informed written consent was taken from every participant of the study.

Use of AI and AI-assisted technologies in the writing process

During the preparation of this work, the author(s) used ChatGPT to correct the language. After using this tool/service, the author(s) reviewed and edited the content as needed and take/s full responsibility for the content of the published article.

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