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Enhancing Diagnostic Accuracy in Bethesda III Thyroid Nodules: The Predictive Value of Serum IL-17A and Calprotectin

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Background: Thyroid nodules are common in clinical practice, with malignancy detected in about 5% of cases. Current risk-stratification approaches, which rely on sonographic features and cytopathological assessment, have notable limitations, especially for indeterminate nodules categorized as Bethesda III.

Aims: To address these diagnostic challenges, this study evaluated the utility of preoperative serum interleukin (IL)-17A and calprotectin levels as potential biomarkers for distinguishing malignant from benign Bethesda III thyroid nodules.

Study Design: This single-center prospective methodological study.

Methods: In this prospective study, 76 patients with Bethesda III nodules scheduled for thyroidectomy were enrolled. Based on histopathological findings, nodules were classified as benign (n = 41) or malignant (n = 35). Comprehensive patient information was collected, including demographics, medical history, and detailed clinical parameters related to nodule characteristics and laboratory results. Circulating biomarkers measured included thyroid-stimulating hormone, free T3, free T4, thyroglobulin (TG), anti-TG, anti-thyroid peroxidase, calcitonin, IL-17A, and calprotectin.

Results: Patients with malignant nodules (n = 35) exhibited significantly higher preoperative IL-17A and calprotectin levels compared with those with benign nodules (n = 41) ($p < 0.001$ and $p = 0.038$, respectively). Receiver operating characteristic analysis demonstrated promising diagnostic performance for IL-17A [area under the curve (AUC) = 0.733] and calprotectin (AUC = 0.639).

Conclusion: IL-17A and calprotectin emerge as promising biomarkers for refining Bethesda III nodule stratification. Incorporating these inflammatory markers into existing diagnostic protocols may substantially reduce unnecessary surgical interventions, thereby alleviating patient anxiety, surgical risks, and healthcare costs.

INTRODUCTION

Advanced imaging techniques have transformed the detection of abnormal thyroid nodules. Approximately 60% of the general population harbors thyroid nodules[1], though only about 5% prove malignant, symptomatic, or progress to functional disease.[1] The majority are benign, asymptomatic, and require no treatment.[2] Despite this, thyroid nodules are often overdiagnosed and overtreated, while thyroid cancer-related mortality has remained unchanged.[3] This suggests unnecessary thyroidectomies continue to be performed without patient benefit, particularly in Asian regions where surveillance protocols for indeterminate nodules remain inadequate.[4]

The Bethesda System is the current clinical standard for classifying thyroid nodules.[5] However, Bethesda III nodules (atypia of undetermined significance/follicular lesion of undetermined significance), which account for 13-30% of cases[4,6], pose a major diagnostic challenge due to their indeterminate nature. Traditional markers-including thyroid-stimulating hormone (TSH), preoperative free T3 and T4[7,8], and circulating biomarkers such as thyroglobulin (TG)[9] and calcitonin[10] are routinely monitored postoperatively but remain insufficient to determine malignancy in Bethesda III cases.[11] Recent investigations have examined non-coding RNAs[11,12], and inflammatory mediators[13] as potential circulating biomarkers for predicting thyroid malignancy.

Among inflammatory mediators, the link between autoimmunity and thyroid cancer is complex. Three pathophysiological mechanisms may underlie this relationship: (I) pre-existing autoimmunity predisposing to malignancy through chronic

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inflammation; (II) antitumor immune responses triggering specific autoimmunity; and (III) immune tolerance permitting malignancy despite autoimmunity. Interleukin (IL)-17A, secreted by T helper 17 cells in response to antigen presentation, has been studied as a prognostic marker in several cancers. Calprotectin and IL-17A-both associated with chronic inflammation-have shown promise in oncology. Calprotectin, a protein linked to inflammatory bowel disease (IBD)[14], has demonstrated prognostic relevance in papillary thyroid carcinoma (PTC)[15] and head and neck cancer.[16] IL-17A, a type III cytokine abundant in the tumor microenvironment, has been correlated with the severity of PTC[17,18], autoimmune thyroiditis,[19] and Hashimoto's disease coexisting with thyroid carcinoma.[20] Despite this evidence, their role as circulating biomarkers for thyroid malignancy remains largely unexplored.

Accurate stratification of malignant thyroid nodules is hindered by the limitations of existing diagnostic methods, including TIRADS and Bethesda classification, in indeterminate cases. A more comprehensive strategy is needed to improve malignancy prediction. Research on IL-17A and calprotectin as circulating biomarkers for thyroid malignancy is limited. Our study evaluated the diagnostic performance of preoperative serum IL-17A and calprotectin levels in stratifying Bethesda III thyroid nodules as benign or malignant, thereby addressing this critical gap in diagnostic approaches.

MATERIALS AND METHODS

Study design and setting

This single-center prospective methodological study was conducted with patients admitted to the endocrinology outpatient clinic at a university hospital during January-September 2023. The Non-Interventional Clinical Research Ethics Committee of the Karabük University approved the study (aprroval number: 2023/1211; date: 13.01.2023). All participants provided their informed consent before their participation in the study. The study followed good clinical practice standards, the Declaration of Helsinki guidelines, and the Standards for Reporting Diagnostic Accuracy Studies.[21]

Sample size and participants

No prior sample size was calculated for this study. We aimed to include all eligible patients in the study. The inclusion criteria were as follows: (1) age ≥ 18 years, (2) diagnosis of multinodular goiter or nodular goiter, and (3) two consecutive fine-needle aspiration cytology (FNAC) results classified as Bethesda III.

A total of 1046 patients with nodular or multinodular goiter were referred to the endocrinology clinic. Patients were excluded (1) who not have two consecutive FNAC results (n = 19), (2) had malignancies classified as other than Bethesda III (n = 380), (3) were using medications and supplements such as anticoagulants (n = 43), vitamin E supplements (n = 7), and/or medications to control cholesterol and lipid metabolism (n = 156), (4) were pregnant (n = 4), (5) were diagnosed with an ongoing infection (n = 18), (6) were diagnosed with an autoimmune and/or autoinflammatory condition (n = 52), (7) were diagnosed with chronic health conditions such as hypertension, renal failure, liver failure, cerebrovascular diseases, or neurodegenerative disorders (n = 62), (8) were diagnosed with any other type of malignancy (n = 13), or (9) were smokers (n = 196). Among patients who were suitable for inclusion in the study, some were eliminated owing to blood sample hemolysis (n = 1), surgical decline (n = 14), refusal to participate in the study (n = 3), and the absence of pathology reports (n = 2).

Data and variables

The demographics, including gender, age, and physicals [i.e., weight, height, body mass index (BMI), and waist and hip circumference], and clinical information, including preoperative sonographic findings and biopsy results, were collected from patients' electronic medical records. Every patient in the study underwent a comprehensive physical examination, and a detailed medical history was obtained. Preoperative sonographic findings of the index nodules were recorded. The index nodule was defined as a "thyroid nodule that requires surgery." This determination was based on the results of preoperative ultrasonography and FNAC results of the nodule. Past FNAC refers to FNAC biopsies performed outside our institution or before enrollment in this study. All patients included in this study underwent two consecutive FNABs at our institution approximately 3 months apart before the surgical decision-making.

Routine biochemical analysis of the blood samples, including free T3 and T4, TSH, TG, thyroid-peroxidase (TPO), IL-17A, and calprotectin was performed before the surgery.

The nodules were classified as benign or malignant based on the histopathological evaluation of the thyroid surgical specimens.[6] The malignant group included PTC, follicular thyroid carcinoma (FTC), follicular tumor of uncertain malignant potential (FT-UMP), and non-invasive follicular thyroid neoplasm with papillary-like nuclear features (NIFTP). Histopathological and clinical characteristics [such as the histopathological type, tumor diameter, tumor localization, multicentricity, tumor-node-metastasis (TNM) staging, clinical stage, age-metastasis-extrathyroidal invasion-size (AMES) risk classification, distant metastasis-age-completeness of resection-local invasion-tumor size risk classification, radioactive iodine treatment] of the patients with malignant nodules were recorded.

The current World Health Organization (WHO) classification categorizes NIFTP and FT-UMP as low-risk thyroid neoplasms; however, the present cases were included in the malignant group as they had undergone identical preoperative evaluation and surgical management protocols as clearly malignant cases and represented clinically challenging scenarios wherein biomarker assistance is most needed.

Ultrasound and biopsy procedures

Ultrasonography was performed by using a conventional device with a 10-MHz linear array transducer (Aloka SSD-4000, Japan). The index nodules were characterized based on the echogenicity, calcification status, vascularity, TIRADS, anteroposterior-to-transverse ratio, and longitudinal diameter.

All biopsies were performed by the same experienced endocrinologist. Consecutive FNACs were performed approximately

3 months apart. FNACs were performed by using a classical method as described by Durante et al.[1]

Sample collection and biomarker analysis

Preoperative blood samples were obtained from all patients. Blood samples were collected into serum-separator tubes. The sera were aliquoted and stored at -80 °C until analysis. The serum levels of IL-17A and calprotectin were quantified by ELISA kits from Elabscience (IL-17A: E-EL-H0105; calprotectin: E-EL-H2357) in strict accordance with the manufacturer's instructions.

Statistical analysis

The IBM SPSS Statistics for Windows version 25.0 (IBM Corp., Armonk, NY, USA) and MedCalc version 16 (MedCalc Software, Ostend, Belgium) were used for statistical analysis. Descriptive statistics were presented as the median with interquartile range for numerical variables and frequency (n) and percentage (%) for categorical variables. Independent-samples Mann-Whitney U test was performed to compare the numerical data between the study groups. Categorical variables were compared between the study groups using the Pearson chi-square test or Fisher's exact test. The diagnostic performance of the routine tests, which were statistically significant in the benign and malignant groups, IL-17A, and calprotectin, was analyzed by receiver operating characteristic (ROC) curve analysis. We selected to dichotomize the biomarker levels using ROC-derived cut-offs to provide clinically actionable thresholds, acknowledging that this approach prioritizes clinical interpretability over statistical power. We selected the Youden J index to determine our optimal cut-off values as it maximizes both sensitivity and specificity together. The ROC curves were compared using Delong et al. method, while Bonferroni correction was applied to adjust pairwise comparisons. Variables that were statistically significant in univariable analyses and certain relevant covariates, such as age, TSH, and anti-TPO, which are biologically plausible confounders, were included in the multivariable logistic regression models by using the forward selection method. We acknowledge that forward selection without cross-validation may lead to overfitting, and these results should be considered exploratory pending validation in independent cohorts. Multicollinearity assessment was performed by the variance inflation factor (VIF) analysis. All variables demonstrated acceptable VIF values (range: 1.046-1.274), well below the concerning threshold of 5.0. The tolerance values ranged from 0.785 to 0.981, all of which were above the acceptable threshold of 0.2. Although the condition index reached 32.6 in one dimension, suggesting minimal multicollinearity, the variance proportions and VIF values indicated this does not represent a concerning level of multicollinearity that would affect model stability or interpretation. Calibration statistics such as pseudo R^2, Hosmer-Lemeshow test, and classification percentages were calculated for the final logistic regression model. $p < 0.05$ was accepted as the statistical significance level.

RESULTS

Study cohort

We compiled the cohort from patients admitted to the clinic during January-September 2023 (Figure 1).

Baseline characteristics

We divided the cohort into benign (n = 41) and malignant (n = 35) nodule groups. The age and gender distributions were comparable ($p > 0.05$). Females predominated in both the groups: benign (n = 35, 85.4%) and malignant (n = 30, 85.7%). A minority of patients in each group reported thyroid-related history, including family history of thyroid cancer (2.4% vs. 5.7%), prior neck radiotherapy (9.8% vs. 5.7%), and previous thyroid surgery (7.3% vs. 2.9%) (all $p > 0.05$). Half of the patients with benign nodules (n = 21, 51.2%) and 34.3% of those with malignant nodules had previously undergone FNAC ($p > 0.05$). While BMI was similar between groups ($p > 0.05$), waist circumference was significantly higher in the malignant group ($p < 0.001$).

Laboratory parameters revealed marked differences. Free T4 levels were significantly lower in malignant compared to benign nodules ($p = 0.037$), whereas preoperative anti-TG ($p < 0.001$), IL-17A ($p < 0.001$) (Figure 2), and calprotectin ($p = 0.038$) (Figure 3) levels were significantly elevated in malignant cases (n = 35) relative to benign (n = 41). Free T3 levels did not differ significantly. The benign group had a higher median number of nodules (5.0) compared to the malignant group (3.0) ($p = 0.004$). Anti-TG positivity was more frequent in malignant nodules (42.9%) than in benign nodules (9.8%) ($p = 0.002$). Furthermore, autoimmune thyroid disease was identified in 18 patients (51.4%) with malignant nodules versus 11 patients (26.8%) with benign nodules ($p = 0.049$) (Table 1).

The sonographic findings of the thyroid nodules

The characteristics of the nodules are presented in Table 2. Sonographic characteristics, including nodule volume, diameter, AP/T ratio, echogenicity, calcification, halo sign, and TIRADS classification, were comparable between the benign and malignant groups ($p > 0.05$).

IL-17A and calprotectin with regard to tumor characteristics

IL-17A ($p = 0.030$) was significantly higher in the anti-TG positive patients than in the anti-TG negative patients. IL-17A ($p < 0.001$) and calprotectin ($p = 0.027$) were significantly higher in patients with PTC and FTC nodules than in patients with benign nodules (Table 3).

The validation of circulating biomarkers

We determined the diagnostic performance of free T4, anti-TG, IL-17A, and calprotectin by using ROC analysis (Table 4; Figure 4). The results showed that free T4 [area under the curve (AUC) = 0.639] and calprotectin (AUC = 0.639) indicated limited diagnostic accuracy, whereas both anti-TG (AUC = 0.718) and IL-17A (AUC = 0.733) revealed acceptable diagnostic accuracy. Overall, IL-17A appeared to be a superior marker compared to free T4 and calprotectin and comparable to anti-TG (Table 4; Figure 4). At these cut-offs, positive predictive values were 81.8% for IL-17A and 85.7% for anti-TG, whereas negative predictive values were 70.8% and 63.8%, respectively. Comparison of the AUC for differentiating malignant nodules is indicated in Table 5. ROC analysis results were interpreted considering the post-hoc statistical power: IL-17A (power: 95.6%),

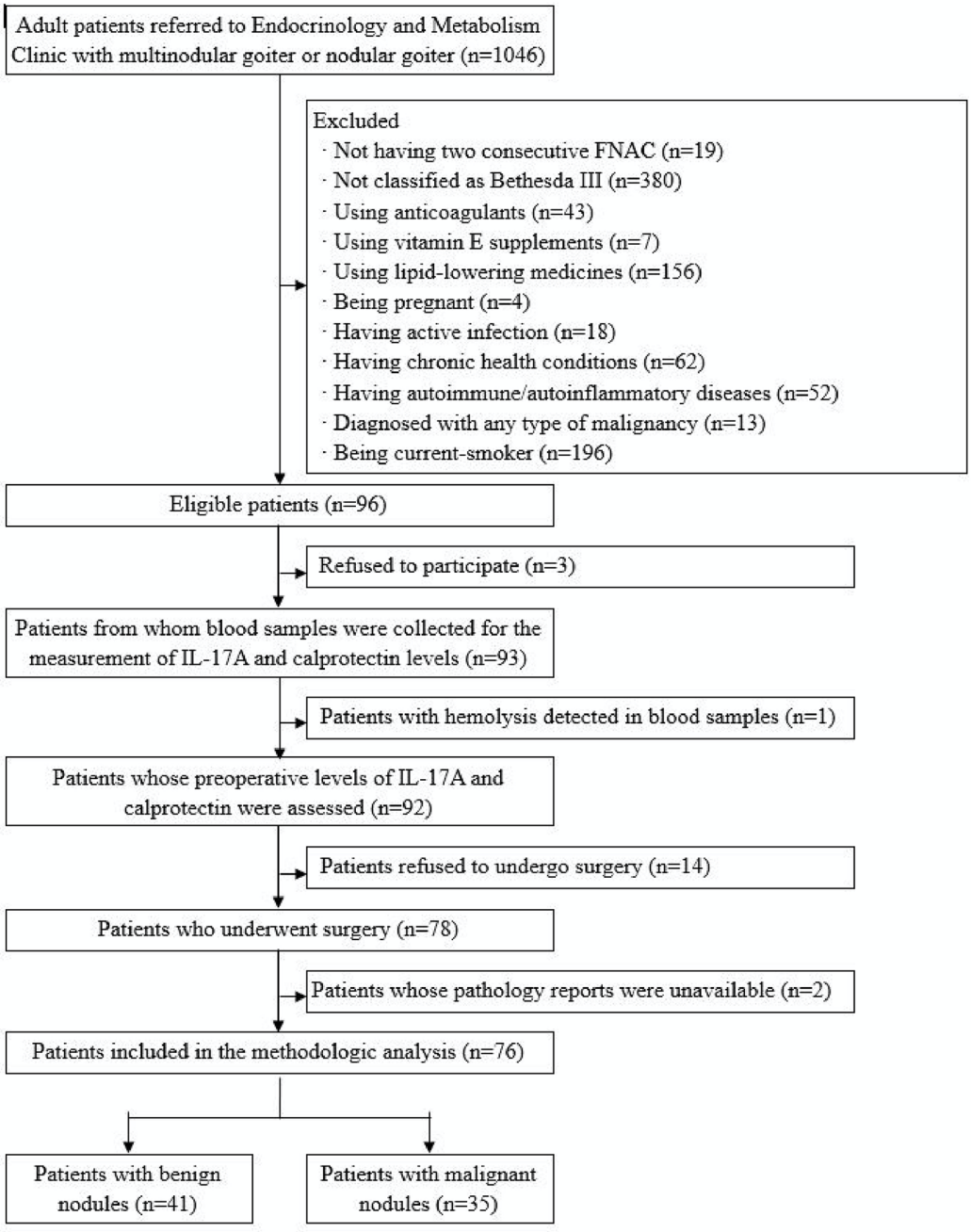

FIG. 1. Flow-chart of the study.
IL-17A, interleukin-17A; FNAC, fine-needle aspiration cytology.

anti-TG (power: 85.2%), calprotectin (power: 57.6%), and free T4 (power: 41.4%).

The association of dependent and independent risk factors of thyroid malignancy

Multivariable logistic regression analysis results indicated that high waist circumference [odds ratio (OR): 1.088, 95% confidence interval (CI): 1.037-1.142, $p = 0.001$], high calprotectin (OR: 1.350, 95% CI: 1.071-1.704, $p = 0.011$), and autoimmune thyroid disease presence (OR: 4.362, 95% CI: 1.322-14.390, $p = 0.016$) were independently associated with the malignant nodules (Table 6; Figure 5). Other variables included in the analysis were age ($p = 0.126$), free T4 ($p = 0.098$), TSH ($p = 0.488$), anti-TG ($p = 0.173$), anti-TPO ($p = 0.234$), IL-17A ($p = 0.095$), and preoperative thyroid nodule number (p

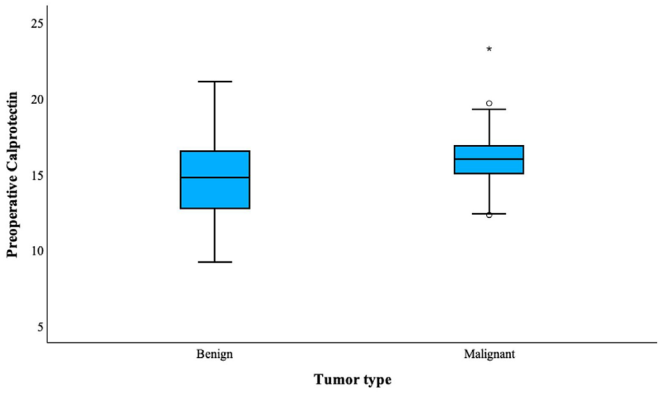

FIG. 2. Comparison of preoperative calprotectin levels between benign and malignant tumors.

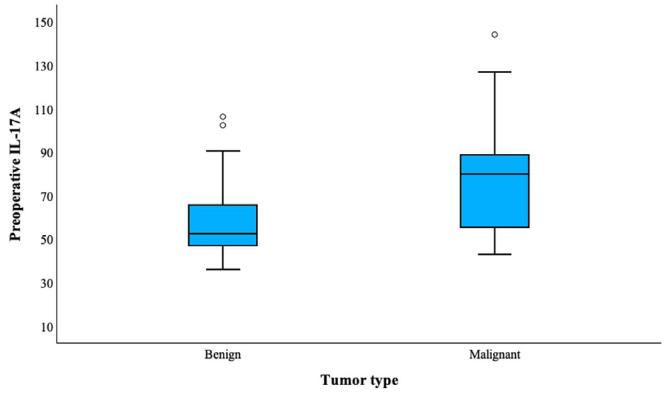

FIG. 3. Comparison of preoperative IL-17A levels between benign and malignant tumors.
IL-17, interleukin-17.

= 0.249), which were found to be non-significant. According to Hosmer-Lemeshow test result (p = 0.228), the final regression model fitted well with our sample. In addition, the performance of the regression model for differentiating malignant nodules was statistically significant (AUC = 0.819, 95% CI: 0.713-0.898, $p < 0.001$). Correct classification percentage for benign nodules was 78.0%, it was 73.5% for malignant nodules, and 76.0% for overall correct classification (Table 6).

Malignant thyroid nodule characteristics

Among the malignant neoplasms, 71.4% of malignancies (n = 25) were PTC. No preference for either the right (51.4%) or left side (45.7%) of the thyroid was detected. Among the malignant cases, 94.5% were classified as stage 1, 5.7% as stage 2, and the majority (74.3%, n = 26) as low risk according to the AMES classification. Among the patients, 48.6% were classified as T_{1a} and 28.5% as T_{1b} of the TNM scale (Table 7).

Post-operative biomarker measurements were available for a subset of malignant cases (n = 21) due to resource constraints. In this limited sample, the IL-17A levels decreased significantly after tumor removal (79.8-70.4 pg/mL, $p < 0.001$), although the levels remained higher than in benign cases (p = 0.007). The calprotectin levels also decreased significantly (15.6-14.8 ng/mL, $p < 0.001$) and became comparable to benign cases (p = 0.795).

DISCUSSION

Our study evaluated preoperative serum IL-17A and calprotectin levels as potential biomarkers for stratifying indeterminate Bethesda III thyroid nodules. Both biomarkers were significantly elevated in malignant compared with benign nodules, with IL-17A demonstrating particularly strong diagnostic potential. These findings suggest that IL-17A, alongside calprotectin, could improve diagnostic accuracy where conventional approaches fail to determine malignancy.

Serum concentrations of thyroid-related hormones (i.e., TSH, free T3, and free T4) are often used to assess thyroid malignancy.[8,9,22] These findings are primarily useful to assess the malignancy of thyroid nodules. Different EU-based[1,23] and US-based[24] TIRADS risk-stratification systems evaluated the four features of nodules: volume, microcalcification, abnormal margins, and echogenicity. Studies comparing the diagnostic performance of different TIRADS systems have shown that they are either comparable[25,26] or that ACR TIRADS is more comprehensive and accurate.[27,28] However, all studies suggest that sonographic finding-based TIRADS risk classification alone is insufficient to predict malignancy and that it should be combined with cytopathological findings.[1,6,26] TIRADS is recommended to select nodules for further cytological FNAC analysis so as to avoid unnecessary biopsies.[26,27] However, this combined system is imperfect: subjectivity among pathologists and insufficient or contaminated samples are the major drawbacks of the cytological analysis.[6,29] In cases where the nodules fall into Bethesda III or IV categories, accounting for approximately 30% and 45% of the malignant cases, respectively[6], and present diagnostic ambiguity, a more comprehensive panel seems beneficial to identify malignant thyroid nodules.

Serological biomarkers are used to determine thyroid abnormalities, including the calcitonin levels for medullary thyroid cancer[30] and thyrotropin for hyperfunctional thyroid adenoma. TG, a thyroid tissue-specific glycoprotein, is also included in serological analysis, but only for long-term follow-up of differentiated thyroid cancer patients who underwent surgery[31] as both normal and abnormal thyroid cells express TG. On the other hand, TG-related antibodies, anti-TG, and anti-TPO, have been primarily associated with thyroid-related autoimmune diseases such as Hashimoto's thyroiditis, Graves' disease, neonatal hypothyroidism, and postpartum thyroiditis.[32] Our analysis revealed that anti-TG is a strong predictor of malignant thyroid nodules (Table 1), with acceptable diagnostic accuracy and high specificity.

IL-17A has been associated with autoimmunity. Research has shown that IL-17, either mRNA or protein levels, was elevated in autoimmune thyroiditis[33], pediatric Hashimoto's disease[34], and PTC.[18,20] In thyroid cancer, cases with elevated IL-17A serum levels were associated with an autoimmune thyroid condition, either Hashimoto's disease[18,20] or lymphocytic thyroiditis.[18]

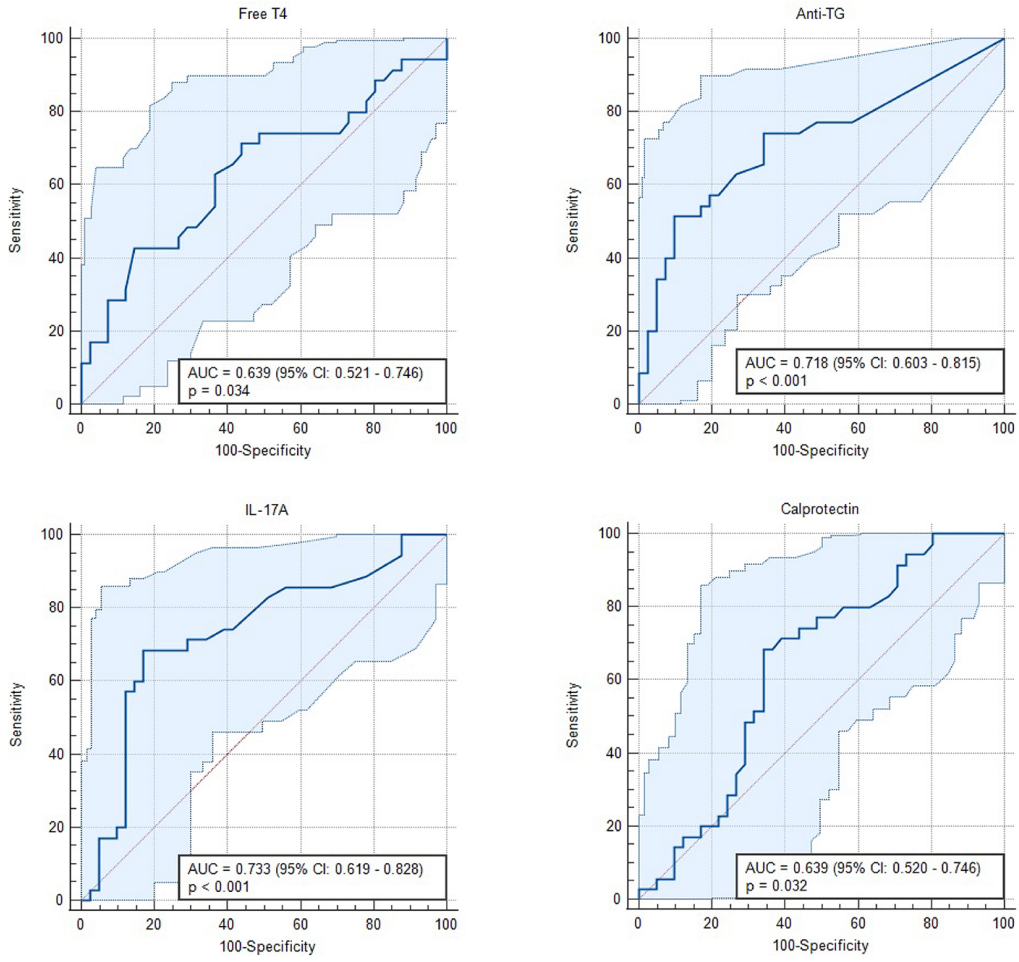

FIG. 4. ROC curves with 95% confidence interval bands of IL-17A, calprotectin, free T4, and anti-TG results for differentiating malignant nodules.
ROC, receiver operating characteristic; TG: thyroglobulin; IL-17, interleukin-17; AUC, area under the curve; CI, confidence interval.

Another study demonstrated that, in the absence of thyroid carcinoma, the IL-17A levels were comparable among patients with Hashimoto's thyroiditis, Graves' disease, and the healthy controls,[35] suggesting that the elevation of IL-17A may be indirectly attributed to thyroid carcinoma. Our findings of elevated IL-17A levels in malignant nodules complement this evidence, emphasizing the direct association between thyroid tumors and increased IL-17A levels. The persistent elevation of IL-17A in malignant cases further suggests a potential role for this cytokine in thyroid cancer pathogenesis, possibly by driving tumor-associated inflammation and immune dysregulation.

Calprotectin is an inflammatory mediator and has been implicated in chronic inflammatory conditions. The increased concentration of calprotectin is associated with IBD[14], rheumatoid arthritis[36], pancreatic carcinoma[37], and subacute thyroiditis[38]; its role in head and neck carcinoma[16] and PTC[15] has been recognized previously. In conformance with the current findings, our results suggested a direct correlation between the calprotectin levels and malignant thyroid nodules. The reduced specificity and limited sensitivity of calprotectin (Table 4; Figure 4) provided a logical explanation, considering that calprotectin is involved in diverse inflammatory conditions, and removing the abnormal tissue reduced the calprotectin levels significantly.[38] Similar to Tabur et al.[15] our findings demonstrated a clear association between the calprotectin levels and thyroid malignancy, suggesting that this inflammatory mediator may serve as a reliable indicator of malignant transformation in thyroid tissues.

Although our findings demonstrated promising diagnostic accuracy for IL-17A and calprotectin, translation to clinical practice warrants a comprehensive evaluation of the clinical utility metrics beyond the conventional sensitivity and specificity measures. Decision curve analysis is deemed essential to determine the optimal risk thresholds where biomarker testing provides a net clinical benefit relative to the default management strategies for Bethesda III nodules. In addition, net reclassification improvement studies should assess how these inflammatory markers enhance the existing risk stratification systems when integrated with TIRADS scoring and cytological findings. In clinical practice, these biomarkers could be integrated into a tiered decision-making process where patients with Bethesda III cytology undergo biomarker testing, and those

TABLE 1. Demographics, Clinical Features, and Preoperative Laboratory Findings of the Patients.

Variables		Patients with benign nodules (n = 41)	Patients with malignant nodules (n = 35)	*p*
Age (year), median (IQR)		52 (45.0-61.5)	49 (43-59)	0.302†
Gender, n (%)	Female	35 (85.4)	30 (85.7)	0.966#
	Male	6 (14.6)	5 (14.3)	
Family history of thyroid cancer, n (%)		1 (2.4)	2 (5.7)	0.592§
Previous neck radiotherapy, n (%)		4 (9.8)	2 (5.7)	0.681§
Previous FNAC, n (%)		21 (51.2)	12 (34.3)	0.138#
Previous thyroid surgery, n (%)		3 (7.3)	1 (2.9)	0.620§
BMI (kg/m^2), median (IQR)		29.7 (26.8-34.2)	30.1 (26.6-33.6)	0.963†
Waist circumference (cm), median (IQR)		102 (92-111)	114 (110-120)	**< 0.001†**
Hip circumference (cm), median (IQR)		112 (104-122.5)	110 (105-114)	0.602†
Thyroid status, n (%)	Hyperthyroid	4 (9.8)	2 (5.7)	0.759§
	Hypothyroid	11 (26.8)	8 (22.9)	
	Euthyroid	26 (63.4)	25 (71.4)	
Free T_3 (pg/mL), median (IQR)		3.34 (3.10-3.52)	3.30 (3.01-3.60)	0.574†
Free T_4 (pg/mL), median (IQR)		1.21 (1.02-1.36)	1.08 (0.97-1.33)	**0.037†**
TSH (µIU/mL), median (IQR)		1.84 (0.85-3.89)	1.64 (1.01-3.70)	0.988†
TG (ng/mL), median (IQR)		41.6 (17.5-162.7)	32.3 (14-154)	0.751†
Anti-TG (IU/mL), median (IQR)		0.2 (0-1)	2.5 (0.1-50)	**< 0.001†**
Anti-TG positivity, n (%)	Negative	37 (90.2)	20 (57.1)	0.002#
	Positive	4 (9.8)	15 (42.9)	
Anti-TPO (IU/mL), median (IQR)		42.1 (28-72.1)	46.5 (34-1300)	0.106†
IL-17A (pg/mL), median (IQR)		52.8 (47.2-66.9)	80 (52.8-89)	**< 0.001†**
Calprotectin (ng/mL), median (IQR)		14.8 (12.6-16.8)	16 (14.9-16.9)	**0.038†**
Elevated calcitonin, n (%)		8 (19.5)	7 (20)	0.999#
Thyroid volume (cc), median (IQR)		19.4 (14.6- 45.3)	21.3 (12.2-55.2)	0.625†
Number of nodules, median (IQR)		5 (3-7)	3 (1-5)	**0.004†**
Multiplicity, n (%)	Solitary	4 (9.8)	9 (25.7)	0.066#
	Multiple	37 (90.2)	26 (74.3)	
Treatment, n (%)	Total thyroidectomy	33 (80.5)	31 (88.6)	0.409§
	Left lobectomy	3 (7.3)	3 (8.6)	
	Right lobectomy	5 (12.2)	1 (2.9)	
Autoimmune thyroid disease, n (%)		11 (26.8)	18 (51.4)	**0.049#**

†Mann–Whitney U test, #Pearson chi-square test, §Fisher's exact test.
IQR, interquartile range; FNAC, fine-needle aspiration cytology; BMI, body mass index; T3, triiodothyronine; T4, thyroxine; TSH, thyroid-stimulating hormone; TG, thyroglobulin; Anti-TG, anti-thyroglobulin antibody; Anti-TPO, anti-thyroid peroxidase antibody; IL-17A, interleukin-17A.

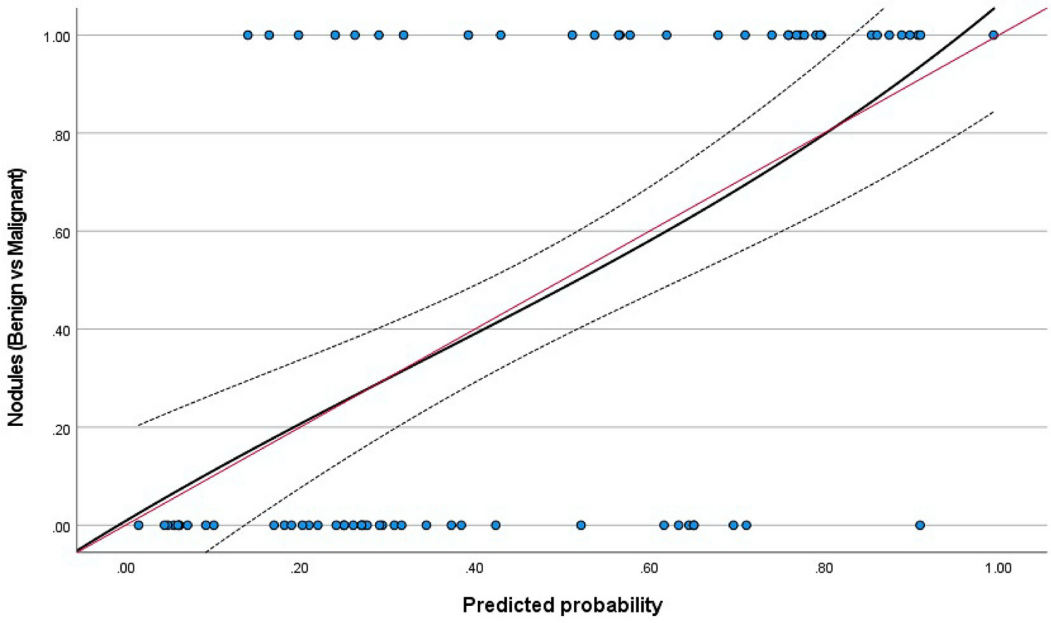

FIG 5. Calibration plot of the multivariable logistic regression model (black curve: fit line, dotted curves: 95% confidence interval of fit line, red line: reference line).

TABLE 2. Preoperative Sonographic Findings of the Index Nodules.

Variables		Benign nodules (n = 41)	Malignant nodules (n = 35)	*p*
Volume (cc), median (IQR)		1.3 (0.7-3.7)	1.2 (0.5-15.1)	0.455†
Longitudinal diameter (mm), median (IQR)		18.9 (13.8-24.5)	18 (12.1-39)	0.770†
AP/T ratio, median (IQR)		0.71 (0.64-0.86)	0.77 (0.67-0.89)	0.229†
Echogenicity, n (%)	Anechoic	0 (0)	1 (2.9)	0.540§
	Hypoechoic	5 (12.2)	5 (14.3)	
	Isoechoic	22 (53.7)	23 (65.6)	
	Iso-hypoechoic	9 (21.9)	5 (14.3)	
	Iso-hyperechoic	3 (7.3)	1 (2.9)	
	Indeterminate	2 (4.9)	0 (0)	
Calcification, n (%)	None	32 (78.1)	29 (82.8)	0.679§
	Macro	3 (7.3)	3 (8.6)	
	Micro	3 (7.3)	1 (2.9)	
	Macro and micro	1 (2.4)	2 (5.7)	
	Eggshell	2 (4.9)	0 (0)	
Halo sign, n (%)		13 (31.7)	15 (42.9)	0.315#
TIRADS, n (%)	1	0 (0)	0 (0)	0.734§
	2	0 (0)	1 (2.9)	
	3	22 (53.7)	18 (51.4)	
	4	17 (41.4)	13 (37.1)	
	5	2 (4.9)	3 (8.6)	

†Mann–Whitney U test, #Pearson chi-square test, §Fisher's exact test.
IQR, interquartile range; AP/T ratio, anteroposterior to transverse diameter ratio; TIRADS, thyroid Imaging reporting and data systems.

with elevated IL-17A (> 71.66 pg/mL) and calprotectin (> 15.55 ng/mL) levels would be prioritized for surgical intervention, while those with lower levels could be considered for active surveillance protocols. Future validation studies should therefore examine predictive values across different clinical populations and pre-test probability scenarios, considering that biomarker performance varies significantly with baseline malignancy rates. Such a comprehensive clinical impact assessment, when combined with clear decision algorithms, is considered crucial for regulatory approval, guideline development, and evidence-based implementation in thyroid nodule-management protocols.

This study has some limitations. First, regarding the study design, this was a single-center, exploratory study with a limited sample size of 76 patients, which may have limited the statistical power and generalizability of our findings. Considering that this was an exploratory investigation intending to assess the potential value of

TABLE 3. Summary of IL-17A and Calprotectin with Regard to tumor Characteristics.

	n	IL-17A (pg/mL), median (IQR)	*p*	Calprotectin (ng/mL), median (IQR)	*p*
Anti-TG status					
Negative	57	52.77 (47.16-79.83)	0.030†	15.44 (14.08-7.00)	0.962†
Positive	19	79.25 (58.40-89.00)		15.63 (13.80-16.36)	
Autoimmune thyroid disease					
No	47	54.64 (47.16-81.40)	0.287†	15.44 (14.08-17.00)	0.835†
Yes	29	67.89 (52.77-84.77)		15.61 (13.80-16.43)	
Treatment					
Total thyroidectomy	64	59.34 (48.10-81.20)	0.583†	15.59 (14.08-16.88)	0.545†
Partial thyroidectomy	12	66.91 (51.84-86.54)		14.42 (12.60-16.84)	
Histopathologic type					
Benign	41	52.77 (47.16-65.96)	< 0.001†	14.81 (12.76-16.54)	0.027†
PTC and FTC	26	80.53 (62.00-88.80)		16.20 (15.44-16.82)	

†Mann–Whitney U test.
IQR, interquartile range; Anti-TG, anti-thyroglobulin antibody; PTC, papillary thyroid carcinoma; FTC, follicular thyroid carcinoma; LRTN, low-risk thyroid neoplasm; IL-17A, interleukin-17A.

TABLE 4. ROC Results of IL-17A, Calprotectin, Free T4, and anti-TG Results for Differentiating Malignant Nodules.

Parameter	AUC (95% CI)	p†	Cut-off	Sensitivity (95% CI)	Specificity (95% CI)	PPV	NPV
Free T4	0.639 (0.521-0.746)	0.034	≤ 1.00	42.9 (26.3-60.6)	85.4 (70.8-94.4)	43.9	84.9
Anti-TG	0.718 (0.603-0.815)	< 0.001	> 12.9	51.4 (34.0-68.6)	90.2 (76.9-97.3)	62.5	85.0
IL-17A	0.733 (0.619-0.828)	< 0.001	> 71.66	68.6 (50.7-83.1)	82.9 (67.9-92.8)	72.4	78.7
Calprotectin	0.639 (0.520-0.746)	0.032	> 15.55	68.6 (50.7-83.1)	65.9 (49.4-79.9)	66.7	67.6

†Delong et al. method.
AUC, area under the curve; CI, confidence interval, T4, thyroxine; Anti-TG, anti-thyroglobulin antibody; IL-17A, interleukin-17A; PPV, positive predictive value; NPV, negative predictive value; ROC, receiver operating characteristic.

TABLE 5. Comparison of Area Under Curves for Differentiating Malignant Nodules.

	p†	Adjusted *p*‡
Free T4 vs. Anti-TG	0.381	1.000
Free T4 vs. IL-17A	0.192	1.000
Free T4 vs. Calprotectin	0.994	1.000
Anti-TG vs. IL-17A	0.853	1.000
Anti-TG vs. Calprotectin	0.410	1.000
IL-17A vs. Calprotectin	0.290	1.000

†Delong et al. method, ‡Bonferroni adjusted *p* values for multiple comparisons.
T4, thyroxine; Anti-TG, anti-thyroglobulin antibody; IL-17A, interleukin-17A.

TABLE 6. Multivariable Logistic Regression Analysis for Differentiating Malignant Nodules.

	β coefficient	Standard error	*p*	OR	95% CI for OR	
Waist circumference, cm	0.085	0.025	0.001	1.088	1.037	1.142
Calprotectin (ng/mL)	0.300	0.119	0.011	1.350	1.071	1.704
Autoimmune thyroid disease, yes	1.473	0.609	0.016	4.362	1.322	14.390
Constant	-14.579	3.676	< 0.001			

Cox and Snell $R^2 = 0.303$, Nagelkerke $R^2 = 0.406$
Hosmer-Lemeshow goodness of fit test; chi-square = 9.632, $p = 0.228$
AUC = 0.819, 95% CI: 0.713 - 0.898, $p < 0.001$
Correct classification percentage for benign nodules = 78.0%
Correct classification percentage for malignant nodules = 73.5%
Correct classification percentage for all nodules = 76.0%
OR, odds ratio; CI, confidence interval; AUC, area under curve.

TABLE 7. Histopathologic and Clinical Characteristics of Malignant Thyroid Neoplasms.

Variables (n = 35)		Values
Histopathologic type, n (%)	PTC	25 (71.4)
	FTC	1 (2.9)
	LRTN	9 (25.7)
Tumor diameter (mm), median (IQR)		10 (5-20)
Tumor localization, n (%)	Right	18 (51.4)
	Left	16 (45.7)
	Isthmus	1 (2.9)
Multicentricity, n (%)		8 (22.9)
TNM staging, n (%)	$T_{1a(m)}N_0M_0$	9 (25.7)
	$T_{1a(s)}N_0M_0$	8 (22.9)
	$T_{1b(m)}N_0M_0$	6 (17.1)
	$T_{1b(s)}N_0M_0$	4 (11.4)
	$T_{2(s)}N_0M_0$	5 (14.3)
	$T_{3a(s)}N_0M_0$	3 (8.6)
Stage, n (%)	1	33 (94.3)
	2	2 (5.7)
	3	0 (0)
	4	0 (0)
AMES classification, n (%)	Low-risk	26 (74.3)
	High-risk	9 (25.7)
MACIS classification, median (IQR)		4.8 (4.1-5.5)
RAI treatment, n (%)		14 (40)

PTC, papillary thyroid carcinoma; FTC, follicular thyroid carcinoma; LRTN, low-risk thyroid neoplasm; IQR, interquartile range; TNM, tumor; node, metastasis; AMES, age; metastasis, extrathyroidal invasion and size risk classification; MACIS, distant metastasis, age, completeness of resection, local invasion, and tumor size risk classification; RAI, radioactive iodine treatment.

IL-17A and calprotectin as biomarkers, no formal power analysis was performed. Testing multiple biomarkers without correction could have increased the false-positive risk. The highly selective inclusion and exclusion criteria, such as the exclusion of patients with common comorbidities, smokers, and those with other malignancies, generated a study population that may not reflect real-world clinical settings where thyroid nodules are typically evaluated. In fact, excluding the common comorbidities may have enhanced biomarker performance beyond real-world settings.

Second, regarding tumor classification, the inclusion of low-risk thyroid neoplasms (NIFTP and FT-UMP) in the malignant group reflects the clinical uncertainty and terminology during the study period, although the current WHO classification categorizes these as low-risk neoplasms rather than malignant tumors. Moreover, excluding Bethesda class IV nodules, a more severe but indeterminate type of nodules, may have influenced our results.

Third, concerning biomarker interpretation, despite excluding patients with clinically apparent autoimmune disorders, the possibility of underlying subclinical autoimmunity remains, which might have influenced the relationships identified in the analysis. Both IL-17A and calprotectin are elevated in subclinical autoimmune thyroid conditions, which potentially contribute to the observed associations with malignancy. The higher prevalence of autoimmune thyroid disease in the malignant group (51.4%) when compared with that in the benign group (26.8%) may have contributed to elevated inflammatory biomarker levels. Furthermore, the type of surgical procedure (total thyroidectomy vs. lobectomy) may have influenced the preoperative biomarker levels, particularly in cases where autoimmune thyroid disease was present in the contralateral lobe.

In addition, several important contextual limitations should be noted. The study population of patients undergoing thyroidectomy for Bethesda III nodules may not represent the broader population of patients with indeterminate thyroid nodules. Although molecular diagnostic approaches are currently advised for Bethesda III nodules to enhance diagnostic precision, these techniques remain inaccessible or financially prohibitive in numerous clinical environments, supporting the potential utility of inflammatory biomarkers as complementary tools.

However, although IL-17A and calprotectin levels showed significant correlation with malignancy, the underlying biological mechanisms were not explored in this study. In addition, advanced assessments of clinical utility, such as decision curve analysis or net reclassification improvement, were not performed. These methods would provide deeper insights into the potential clinical impact but require larger, more diverse patient cohorts. Addressing these gaps will be an important objective for future validation studies.

Furthermore, the present findings were not validated in an independent external patient cohort. Therefore, IL-17A and calprotectin cannot be considered as biomarkers for routine clinical application. Post-operative biomarker measurements were only available for a subset of malignant cases (n = 21) owing to resource constraints. Future multicenter studies with larger patient groups are therefore required to confirm and extend these preliminary results before incorporating these biomarkers into clinical decision-making processes. Finally, as this was an exploratory study without formal power calculation, the present statistical findings should be interpreted as hypothesis-generating rather than definitive. The observed associations require validation in larger, independent cohorts before clinical implementation can be considered.

Our findings highlight several practical clinical applications of these biomarkers. Measurement of IL-17A and calprotectin following indeterminate biopsy results may help reduce diagnostic uncertainty and potentially spare patients from unnecessary surgery. The cut-off values identified in our study (IL-17A > 71.66 pg/mL, calprotectin > 15.55 ng/mL) represent preliminary thresholds optimized to balance sensitivity and specificity. Although dichotomization facilitates clinical translation, future research should aim to validate these cut-offs in larger cohorts and evaluate continuous variable modeling to enhance predictive accuracy and account for potential non-linear associations. Combining these inflammatory markers with existing imaging and cytology tools (TIRADS and Bethesda systems) may enhance overall diagnostic accuracy. However, these preliminary findings require validation in larger, multicenter cohorts before clinical application can be considered. Although our statistical modeling approach followed accepted guidelines for exploratory studies, it too requires confirmation in independent patient populations. Future research should incorporate cross-validation techniques and evaluate model performance across diverse cohorts to ensure robustness and generalizability.

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Informed Consent: All participants provided their informed consent before their participation in the study.

Data Sharing Statement: The datasets analyzed during the current study are available from the corresponding author upon reasonable request.

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