A Novel Score Based on Galectin-3 and Tissue Inhibitor of Metalloproteinase-1 Improves the Diagnostic Accuracy of Thyroid Cancer

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Abstract: The invasive fine needle aspiration (FNA) biopsy remains the base investigative tool for thyroid malignancy despite its limitations. This study aimed to evaluate whether serum galectin-3 (Gal-3) or tissue inhibitor of metalloproteinase-1 (TIMP-1) could be used as serum biomarkers for improving thyroid cancer diagnosis. Using ELISA, Gal-3 and TIMP-1 were identified in the sera of 90 Egyptian patients comprised of 39 thyroid cancers, and 51 benign thyroid tumors. Further, multiple regression analyses and ROC curve analyses were applied to create a diagnostic score for thyroid cancer diagnosis. TIMP-1 was reduced significantly in thyroid cancer than in benign tumors ($P < 0.001$), but Gal-3 was slightly increased in thyroid cancer patients ($p=0.391$). TIMP-1 produced 0.848 of area under curve (AUC), plus 83% sensitivity and 75% specificity, while Gal-3 yielded 0.664 AUC with 72% sensitivity and 55% specificity for identifying thyroid cancer. The score combining TIMP-1, Gal-3, FNA, and ultrasound (US) was then constructed yielding 0.898 AUC for thyroid cancer detection with 89% sensitivity and 82% specificity. For the first time, combining serum markers of TIMP-1 and Gal-3 with regular methods of FNA and US, provides superior accuracy for thyroid cancer diagnosis and proper management.

Keywords: Serum marker; Thyroid cancer; Galactin-3 (Gal-3); Tissue inhibitor of metalloproteinase-1 (TIMP-1).

1 Introduction

Thyroid cancer is the most occurring endocrine cancer [1]. It is a highly growing cancer that affected nearly 64,300 new cases in 2016 with an annural rate of 4.5% according to the National Cancer Institute [2]. Its incidence has increased dramatically in the last 3 decades making it the eighth most common cancer in western countries [3]. In Egypt, it accounts for 1.5% of the entire cancers and represents one third of all endocrine cancers. The ratio of affected Egyptian females to males is nearly 3:1 [4]. Consistently, the main factors contributing for thyroid cancer are radiation exposure, family history, and genetics [5]. Papillary thyroid cancer (PTC) is the most widespread type, representing 80–85% of thyroid cancers, follicular thyroid cancer (FTC) represents 10–15%, medullary thyroid carcinoma (MTC) accounts for 3–4%, while anaplastic carcinoma (ATC) is rare [6]. Until now, the key diagnostic tool for thyroid malignancy is fine needle aspiration (FNA) cytology. FNA is invasive, relatively risky, influenced by the cytopathologist, and it may have sampling errors. In addition, it has low sensitivity to diagnose the minimally invasive follicular thyroid tumors which overlap with other thyroid tumor types [7], [8]. Unfortunately, patients with these undefined tumors were diagnosed only after thyroidectomy and about 75% of them were proved to have benign neoplasms [5].

Many advanced studies have turned to use blood markers for widely improving the diagnosis of thyroid cancers and reducing the need for surgical intervention, e.g. calcitonin, thyroglobulin, angiopoietin-1 (Ang-1), chitinase 3-like 1 (YKL-40), cytokeratin 19 (CK19), galectin-3 (Gal-3), and tissue inhibitor of metalloproteinase -1 (TIMP-1) [5], [9], [10], [11]. One of the most effective markers and the most investigated molecule for diagnosing thyroid cancers is Gal-3. It is a protein with higher affinity for β-galactoside residues of glycoproteins on the cell surface and has been found in both cytoplasm and nuclear compartments [12]. On the other hand, matrix metalloproteinases (MMPs) which enhance extracellular matrix (ECM) degradation are controlled by tissue inhibitor of metalloproteinases (TIMPs). Particularly, TIMP-1 acts mainly as inhibitor of MMP-9. Moreover, it functions as inhibitor of angiogenesis, invasion, and metastasis as well as antiapoptotic [13], [14].

This study was established to assess the diagnostic performance of Gal-3 and TIMP-1 in thyroid cancer. Moreover, our goal was to develop a novel score based on TIMP-1 and Gal-3 together with FNA and ultrasound (US) for getting superior efficacy in distinguishing thyroid cancers from benign tumors.

2 Patients and Methods

2.1 Patients

Overall, 90 coherent Egyptian thyroid patients who were operated upon between January 2016 to December 2016 from the department of surgery, Mansoura University Hospitals, Mansoura, Egypt and Ismailia Teaching Oncology Hospitals, Ismailia, Egypt, were enrolled in this study prospectively. Written consents were signed by all participants and the study

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was permitted by the Institution Review Board of the National Cancer Institute. Our selective criteria involved thyroid surgery and postoperative pathological examinations in the hospitals. Furthermore, all patients who were subjected to preoperative evaluations by ultrasound and cytological examinations were classified into two major groups after post-operative pathological evaluation, malignant group (n=39) and benign group (n=51).

2.2 Samples

Venous blood samples were withdrawn from the patients along with the diagnosis period before any surgical operation. The sera were aliquoted into two microcentrifuge tubes. The first aliquot was used for routine thyroid function tests including TSH, free thyroxine (FT4), free triiodothyronine (FT3), total thyroxine (T4), and total triiodothyronine (T3), which were analyzed using Mini Vidas; Biomerieux-Diagnostics, enzyme linked fluorescent immunoassay (ELFA). The second aliquot was stored at -20 °C and thawed only before analysis.

2.3 Preoperative neck/thyroid ultrasound

Ultrasound results were subdivided into two classes: suspicious and non-suspicious. Suspicious ultrasounds were recorded if there were two or more of the malignant features including microcalcifications, irregular margin, hypoechogenicity, solid composition, increased internal vascularity, and pathological lymph nodes.

2.4 Fine needle aspiration biopsy

First, FNA results were categorized into six groups depending on the Bethesda classification system [15], then, were reconstructed into suspicious and non-suspicious categories. The non-suspicious group included all benign cytologic findings, whereas the suspicious group consisted of atypia of undetermined significance (AUS) / follicular lesion of undetermined significance (FLUS), follicular neoplasm (FN) / suspicious for follicular neoplasm (SFN), suspicious for malignancy, and patients diagnosed with malignant cytology.

2.5 Postoperative surgical pathology

Histopathological results were categorized into benign or malignant groups; the latter was further staged using TNM system, where (T) refers to tumor size and local invasion, (N) refers to nodal metastases, and (M) refers to distant metastases [16].

2.6 Quantitation of TIMP-1 and Gal-3 using ELISA

Based on Sandwich-ELISA technique, Gal-3 was analyzed according to the manufacturers' instructions of Elabscience Biotechnology Co., Ltd; (Catalog No: E-EL-H1470; Wuhan, Hubei, China). ELISA kits for TIMP-1 were purchased from Elabscience Biotechnology Co., Ltd (Catalog No: E-EL-H0184; Wuhan, Hubei, China). The optical density (OD value) was determined at once, using a microplate reader (Tecan Infinite F50 Austria GmbH, Austria) set to 450 nm, which is proportional to the concentration of both TIMP-1 and Gal-3.

2.7 Data analysis

Statistical analyses were carried out via a statistical software package SPSS 22.0 for Microsoft Windows (SPSS Inc.). Frequency and percent distribution were used to describe categorical variables whereas quantitative/continuous variables were described as mean ± SD. Comparison of categorical factors (across outcome groups; i.e., malignant vs. benign) was made by the chi-square test, while quantitative continuous variables were analyzed by the Student t-test. The correlation was evaluated by Pearson's correlation coefficient. Statistical significance was set at $P \leq 0.05$ with a 95% confidence interval. In order to assess the diagnostic value of each TIMP-1, Gal-3, FNA, and US, an exclusion criterion was applied on all patients who had FNA with insufficient or unsatisfactory cellular yield so that a total of 80/90 patients were only enrolled with 44 (55%) benign and 36 (45%) malignant thyroid patients. Moreover, by plotting the area under the ROC curves (AUC), the best cut-off values were determined for both Gal-3 and TIMP-1 blood markers. For the multivariate analysis, regression models were established, using different combinations of the following diagnostic tools as TIMP-1, Gal-3, FNA, and US. Further, ROC curve analysis was established for all combinations with the purpose of deciding which one was the most accurate in detecting thyroid carcinoma. Finally, the diagnostic accuracy was calculated by sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV).

3 Results

3.1 Patient's characteristics

The clinical characteristics of benign and malignant thyroid patients are illustrated in Table1. The mean age at diagnosis was 44 years (range 20 to 79), with a female predominance (75.6% vs. 24.4%; female: male ratio, 3:1). The cytological diagnosis included non-diagnostic or unsatisfactory of 11.1%, benign of 42.2%, and malignant of 12.2%. Interestingly, FNA reports included intermediate cytology results which composed of suspicious for malignancy, AUS/FLUS, and FN/SFN of 34.5%. Only 17 (54.8%) of these indeterminate cases were diagnosed as malignant by histopathological examination.

Final histopathology reported that from a total of 90 patients, 51 patients (56.6%) had benign lesions and 39 patients (43.3%) had malignant tumors. The fifty-one patients with benign lesions included 29 (32.2%) nodular goiter, 11 (12.2%) follicular adenoma, 6 (6.7%) thyroiditis, 4 (4.4%) were free from tumor tissue, and 1(1.1%) hyperplastic nodule. The thirty-nine malignant diagnoses comprised papillary carcinoma which was the major type in 28 patients (31.1%) (7 micropapillary carcinoma, 21 papillary carcinoma) and it was subdivided into 23 classic PTC, 4 follicular variant PTC, and 1 tall cell variant. The remaining malignancies included 6 cases (6.7%) of follicular carcinoma, 2 (2.2%) of medullary carcinoma, 2 (2.2%) of poorly differentiated carcinoma, and 1 (1.1%) case of well- differentiated of uncertain malignant potential.

3.2 Measurement of candidate markers

As presented in Table 1, the mean levels of TIMP-1(ng/mL)

TABLE 1
CLINICOPATHOLOGIC CHARACTERISTICS OF BENIGN AND THYROID CANCER PATIENTS (N=90)

Variable	Benign (N=51)	Malignant (N=39)	*x^2; P value
Age (year)	42.2 ± 12.0	46.6 ± 12.9	0.097
Sex			
Male	10 (19.6)	12 (30.8)	1.49; 0.22
Female	41 (80.4)	27 (69.2)	
Thyroid function tests			
TSH (mIU/L)[a]	1.6 ± 1.1	1.7 ± 1.0	0.63
FT4 (pmol/L)[a]	14.9 ± 4.5	16.0 ± 3.8	0.42
FT3 (pmol/L)[a]	5.0 ± 1.1	4.7 ± 1.1	0.57
T4 (nmol/L)[a]	116 ± 27.8	111 ± 22.5	0.66
T3 (nmol/L)[a]	2.3 ± 0.7	1.8 ± 0.4	0.09
Ultrasound			
Non Suspicious	35 (68.6)	15 (38.5)	8.14; 0.004
Suspicious	16 (31.4)	24 (61.5)	
Fine needle aspiration			
No diagnostic or Unsatisfactory	8 (15.7)	2 (5.1)	
Non Suspicious	28 (54.9)	10 (25.6)	14.2; 0.001
Suspicious	15 (29.4)	27 (69.2)	
Markers			
Gal-3 (ng/mL)[b]	6.4 ± 2.2	6.9 ± 2.8	0.391
TIMP-1 (ng/mL)[b]	83.2 ± 10.7	73.1 ± 7.7	<0.001

Values are expressed as mean ± SD or number (%)

[a]*Normal values for thyroid function tests : thyroid stimulating hormone (TSH) 0.4 – 5.8 mIU/L; free thyroxine (FT4) 10–23 pmol/L; free triiodothyronine (FT3) 3.1–7.7 pmol/L; total thyronine (T4) 60–160 nmol/L; total triiodothyronine (T3) 0.9–2.8 nmol/L.*

[b] *Abbreviations: Gal-3: galectin-3, TIMP-1: tissue inhibitor of metalloproteinase-1. *x^2 represents chi-square for qualitative data; p value for qualitative and quantitative data; p >0.05 is considered non-significant; p <0.05 is considered significant.*

decreased in thyroid cancer patients (73.13±7.74) as compared with benign ones (83.25±10.72) giving a highly significant difference between the two patient groups (p <0.001). The mean levels of Gal-3 (ng/mL) were found to be slightly overlapped (p=0.391) between the higher level cancer group (6.92±2.82) and the lower level benign group (6.46±2.21) as shown in Fig. 1.

3.3 ROC curves of candidate markers

Using ROC curve, the diagnostic accuracy of both TIMP-1 and Gal-3 were assessed and compared as biochemical markers of thyroid cancer. The ROC curve was carried out on the selected 80 patients. The areas under the curves for TIMP-1 were (AUC=0.848) with a highly significant p-value (p <0.0001), while Gal-3 had (AUC=0.664); (p =0.012); Fig. 2. Therefore TIMP-1 was so efficient that it was chosen as a basic marker to combine with other markers for discriminating patients with benign versus malignant thyroid diseases.

The cut-off values with the best efficiency of TIMP-1 and Gal-3 were 79 ng/mL, and 6.7 ng/mL, respectively, for distinguishing between positive and negative results.

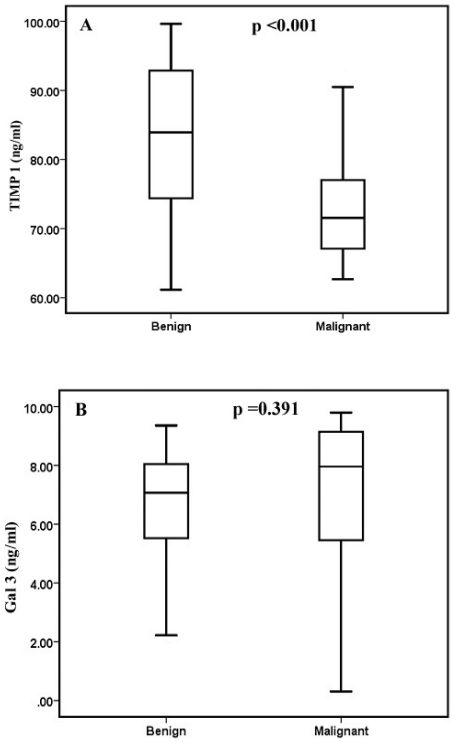

Fig. 1. Box plots of TIMP-1 and Gal-3 blood markers for discrimination malignant thyroid patients from benign subjects. A. Box plots of TIMP-1. B. Box plots of Gal-3. The box represents the interquartile range. The whiskers indicate the highest and lowest values, and the line across the box indicates the median value. p <0.05 is considered significant.

3.4 Predictive model and its performance characteristics

Biochemical markers (Gal-3 and TIMP-1) assessed in the present study were combined with US and FNA by the multiple regression analyses to create several predictive models of thyroid carcinoma. It should be noted that we found no correlation between the two markers, (r=_0.08; p =0.432) in patients with benign and malignant thyroid diseases. Thus, each diagnostic test independently provides different information for the detection of thyroid cancer and, therefore, it was expected to increase the diagnostic accuracy if they were combined together. The optimal multivariable model was selected as having the largest AUC by ROC analysis. Accordingly, a novel model comprising TIMP-1, Gal-3, FNA, and US was the most favorable one (Table 2). It provided the highest AUC [95% confidence interval (CI)] for the prediction of thyroid malignancy [0.898 (0.832–0.965)], as shown in Fig. 2. The score of best fit model for identifying thyroid cancers was [1.78 (Numeric constant) + Gal-3 (ng/mL) × 0.026 + FNA × 0.25 + US × 0.077 − TIMP-1 (ng/mL) × 0.025]. The score (range 1.17–2.05) has a superior significant difference between the two groups of interest (P <0.0001), (Fig. 2). The mean ± SD of score in benign and malignant patients were 0.24±0.27 and 0.7±0.22, respectively. A central cutoff point of 0.51 was selected (i.e. less than 0.51 indicated benignity and ≥0.51 indicated thyroid malignancy) with the highest sensitivity of 89% and highest

TABLE 2

MULTIPLE REGRESSION MODEL FOR THE PREDICTION OF THYROID CANCER

[a] *SE = Standard error.*

Variable	Coefficients	SE[a]	t[b]	P value[c]
US	0.077	0.093	0.831	0.409
FNA	0.25	0.090	2.787	0.007
Gal-3	0.026	0.017	1.513	0.135
TIMP-1	-0.025	0.004	-6.162	<0.001

[b] *t = test value*

[c] *p >0.05 is considered non-significant; p <0.05 is considered significant*

specificity of 82%. While the sensitivities of each individual investigative tool including US, FNA, Gal-3, and TIMP-1 were 64%, 72%, 72%, and 83%, respectively, their specificities were 68%, 64%, 55%, and 75%, respectively (Table 3). All calculated sensitivities, specificities, diagnostic accuracy, and positive or negative predictive values for the investigated markers at the optimal cutoff are found in Table 3.

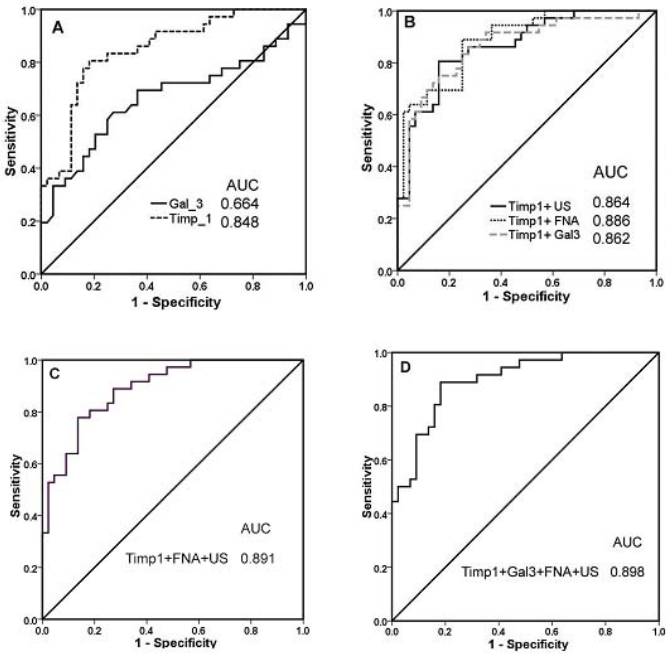

Fig. 2. ROC curves for candidate markers for discriminating patients with thyroid cancer from benign patients. A. ROC curve of Gal-3, and TIMP-1. B. ROC curve of TIMP-1+US, TIMP-1+FNA, TIMP-1+ Gal-3. C. ROC curve of TIMP-1+FNA+US. D. ROC curve of TIMP-1+ Gal-3+FNA+US. AUC: Area under ROC Curve

TABLE 3

DIAGNOSTIC VALUES OF SINGLE AND COMBINED MARKERS FOR THROID CANCER PATIENTS

Evaluated Test	AUC	Cut off value	Sensitivity %	Specificity %	Efficiency %	PPV %	NPV %
Single Variable							
TIMP-1	0.848	79	83	75	79	73	85
Gal-3	0.664	6.7	72	55	63	57	71
FNA	-	-	72	64	68	62	74
US	-	-	64	68	66	62	70
Two Variables							
TIMP-1, Gal-3	0.862	0.45	89	68	78	70	88
TIMP-1, FNA	0.886	0.48	89	75	81	74	89
TIMP-1, US	0.864	0.41	83	75	79	73	85
Three Variables							
TIMP-1, FNA,US	0.891	0.45	89	73	80	73	89
Four Variables							
TIMP-1, Gal-3,	0.898	0.51	89	82	85	80	90

AUC: Area under ROC Curve, PPV: positive predictive value, NPV: negative predictive value.

4 Discussion

Although FNA is considered the principle method for diagnosing thyroid neoplasms, it has several limitations particularly in differentiating between follicular carcinomas and the more predominant follicular adenomas. Search for simple, sensitive, noninvasive, reproducible and effective markers are urgently essential for an accurate diagnosis of thyroid malignancies, meanwhile no validated blood marker is available for thyroid cancer detection [12]. Gal-3 and TIMP-1 have given great interest for their potential role in predicting thyroid cancer. Indeed, almost all studies investigating Gal-3 or TIMP-1 levels in thyroid carcinomas used immunohistochemical examinations, and mRNA measurements, while very little studies were concerned with serum levels [12]. Therefore, this study was designed to investigate serum levels of Gal-3 and TIMP-1 in thyroid cancers. Their diagnostic accuracy in combination with other diagnostic methods like US and FNA was then evaluated.

With respect to Gal-3, it is synthesized primarily in the cytoplasm transferring to other sites as the cell nucleus, cell surface or extracellular space. Cytoplasmic Gal-3 acts as an apoptosis inhibitor, while nuclear Gal-3 promotes mRNA splicing and apoptosis. Clearly, lack of nuclear Gal-3 and its increase in the cytoplasmic compartments enhance tumor progression, while extracellular Gal-3 binds to extracellular matrix glycans which in turn enhances tumor invasion and metastasis [17].

Immunohistochemical studies have emphasized that Gal-3 is overexpressed in thyroid malignancies compared to benign tumors or in normal thyroid tissues [18]. Therefore, serum levels of Gal-3 were supposed to be a helpful tool in distinguishing thyroid cancer from benign tumors preoperatively. Hence in this study, the mean values of serum Gal-3 were investigated in malignant thyroid neoplasms compared to benign neoplasm. The results revealed that the levels of Gal-3 in patients with thyroid cancer were elevated, but with nonsignificant difference with benign patients. Accordingly, these findings agreed with those results obtained by several studies [5], [19], [20], [21], while others [12], [22] observed that serum Gal-3 levels had a significant difference between benign and malignant thyroid masses.

Regarding TIMP-1, it was reported that its expression in PTC was higher than normal tissues, correlating with advanced tumor stage. However, it was opposed to the role of TIMP-1 as inhibitor of invasion and metastasis. Nevertheless, decreased TIMP-1 expression was only found in recurrent thyroid patients relative to non-recurrent patients [5], [23]. Studies concerned with serum TIMP-1 in thyroid cancers are few and contradictory. In a study conducted by Zhou et al. [24], they proposed that serum TIMP-1 levels in papillary thyroid cancer patients were significantly higher than those of benign lesions. In contrast, Makki et al. [5] found no difference between the two same groups. Herein, in the present study, the mean values of serum TIMP-1 levels (ng/mL) were significantly lower ($p<0.001$) in cancer patients (73.13±7.74) than in patients with benign lesions (83.25±10.72), thus pointing to its apparent role in thyroid cancer progression. Overall, these findings suggest that serum TIMP-1 could be an effective blood marker for diagnosing thyroid cancer. Also, the predictive score in the study was by far the first to combine investigative tools as US and FNA with serum biomarkers for the detection of thyroid malignancy preoperatively.

In this research, the sensitivity of US for malignant nodules (64%) was inferior to FNA, Gal-3, and TIMP-1, but comparable with the reported sensitivity in literature which ranged from 46% to 86.5% [25]. Different from this, standard FNA yielded relatively high sensitivity (72%) in our study, which is similar to prior studies with sensitivity of 61% to 97.7% [25]. FNA results had about 45.6% (41/90) of non-diagnostic and indeterminate specimens, which is slightly higher than that reported in previous studies (10% to 40%), where only 46.3% (19/41) were found to be malignant after surgery [26]. Since no single thyroid cancer biomarker has adequate sensitivity and specificity, this research has turned to combine blood markers with simple investigative tools for improved efficiency of thyroid cancer detection. Accordingly, our novel score including TIMP-1, Gal-3, FNA, and US improved AUC value for detecting thyroid cancer from 0.664 for Gal-3 and 0.848 for TIMP-1 to 0.898. Also, the sensitivity of the novel score was significantly higher than the sensitivities produced by each diagnostic tool separately. Hence, the combined score increased sensitivity to 89 %, specificity to 82 %, and efficiency to 85 % for distinguishing malignant from benign thyroid disease. Moreover, this score presented a superior AUC than other combinations in previous studies. Huang et al. [27] used ELISA to detect serum VEGF-C, VEGFR-3 and TSH combination and showed an AUC of 0.862. Tomei et al. [8] investigated combined KIT, CDH1, LSM7, C21orf4, DDI2, TC1, Hs.296031 and BRAF genes in FNA cytological samples using quantitative PCR and showed an AUC of 0.88. Paunovic et al. [28] evaluated molecular thyroid tumor markers: thyroid peroxidase (TPO), and HBME-1 in combination with immunohistochemistry in thyroid tissue sections which yielded an AUC value of 0.786.

4 Conclusion

To date, this is the first study to assess the diagnostic value of a score based on combining TIMP-1, Gal-3, FNAC, and US to improve thyroid cancer identification and potentially avoid unnecessary surgeries. In the future, studies evaluating our score in large patient cohorts are required for the development and validation of a clinically applicable test.

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