RESEARCH ARTICLE

Combined prognostic value of preoperative serum thyrotrophin and thyroid hormone concentration in papillary thyroid cancer

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Abstract

Background: A growing number of studies have found a close association between thyroid hormones and thyrotrophin (TSH), and they also have prognostic significance in some cancer types; this study aimed to investigate the prognostic value of free triiodothyronine (fT3), free thyroxine (fT4), fT3/fT4, TSH, and their combination in patients with papillary thyroid carcinoma (PTC).

Methods: This study retrospectively analyzed the relevant data of 726 newly diagnosed PTC patients. Both univariate and multivariate analyses were used to predict the recurrence rate, and a risk score was established. In addition, with the use of a random survival forest, a random forest (RF) score was constructed. After calculating the area under the curve (AUC), the diagnostic efficacy of risk score, RF score, and four indicators was compared.

Results: fT3, fT4, fT3/fT4, and TSH were strongly associated with some invasive clinicopathological features and postoperative recurrence. Patients with high expression of fT4 and TSH have a high risk of recurrence. By contrast, patients with high expression of fT3 and fT3/fT4 have a low risk of recurrence. At the same time, the combined use of various indicators is more helpful for establishing an accurate diagnosis. By comparison, we found that the RF score was better than the risk score in terms of predicting the recurrence of PTC.

Conclusion: The diagnostic accuracy of a combination of fT3, fT4, fT3/fT4, and TSH can help improve our clinical estimate of the risk of recurrent PTC, thus allowing the development of a more effective treatment plan for patients.

KEYWORDS
fT3, fT4, papillary thyroid carcinoma, prognostic model, TSH

1 | INTRODUCTION

Thyroid cancer is the most common cancer affecting the endocrine system, which accounts for more than 10% of malignant tumors[1]. Its incidence rate is much higher than that of other head and neck tumors. Recently, the incidence of thyroid cancer continues to increase. Currently, it is the fifth most common malignancy in women. Moreover, it is expected to become the second most common malignant tumor in women and the ninth most common malignant tumor in men by 2030[2]. For a long time, differentiated thyroid carcinoma (DTC) has always been a hot topic for clinicians and researchers. There are several types of DTC, including papillary thyroid cancer (PTC), follicular thyroid cancer (FTC), and so on. Among them, PTC has the highest incidence[3]. It is generally associated with a favorable survival prognosis, with less than 2% mortality at 5 years[4]. Therefore, the factors associated with PTC recurrence warrant further investigation.

Thyrotrophin (TSH) is a widely known stimulant of thyroid cells. It is one of the hormones secreted by the anterior pituitary gland; it primarily controls and regulates the thyroid activity. Numerous studies have shown that serum TSH can be used as an independent predictor of thyroid cancer, and higher serum TSH levels are associated with the development of PTC[5]. In addition, other studies have shown that the high expression of TSH usually increases the risk of recurrence in DTC patients[6]. The effect of TSH on the progression of PTC is related to its downregulation of p53 expression[7], but the specific mechanism still needs to be further elucidated. Thyroid hormones, mainly thyroxine (T4) and triiodothyronine (T3), are synthesized and secreted by the thyroid gland. It plays an important role in cancer proliferation, apoptosis, invasion, and angiogenesis. Through several non-genomic pathways, it mediates its action on cancer cells including the activation of plasma membrane receptor integrin $\alpha V\beta3$[8]. Free triiodothyronine(fT3) and free thyroxine(fT4) are the physiologically active forms of T3 and T4, respectively, and can only enter the target cells when they are in a free state to play an active role. fT3/fT4 is also of great significance in judging the thyroid function status. As one of the factors affecting the internal environment of the body, thyroid hormone plays an important physiological role in several cancer types. The study of Nisman B et al. showed that fT4 and fT3/fT4 were valuable in determining the prognosis of breast cancer[9]. The study of Pan JJ et al. showed that the reference value of fT3 is used in the prognosis of thyroid cancer in children and adolescents[10]. To date, most studies that investigated the effect of thyroid hormone in PTC only used a single indicator. However, the combined effect of multiple indicators has not received much attention as well as the interaction among them.

The current study was the first to perform a combined analysis of the role of TSH, fT3, fT4, and fT3/fT4 in the recurrence of PTC. The machine learning method was used to construct the model for predicting recurrence. To further verify the predictive effect of our model, the patients were randomly divided into training set and testing set. This study aimed to explore the comprehensive predictive effect of various indicators, especially TSH and thyroid hormone, on PTC.

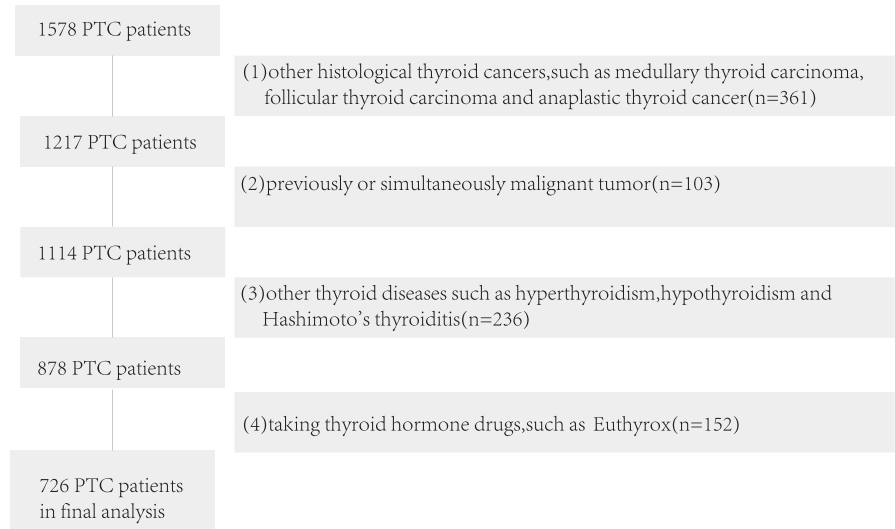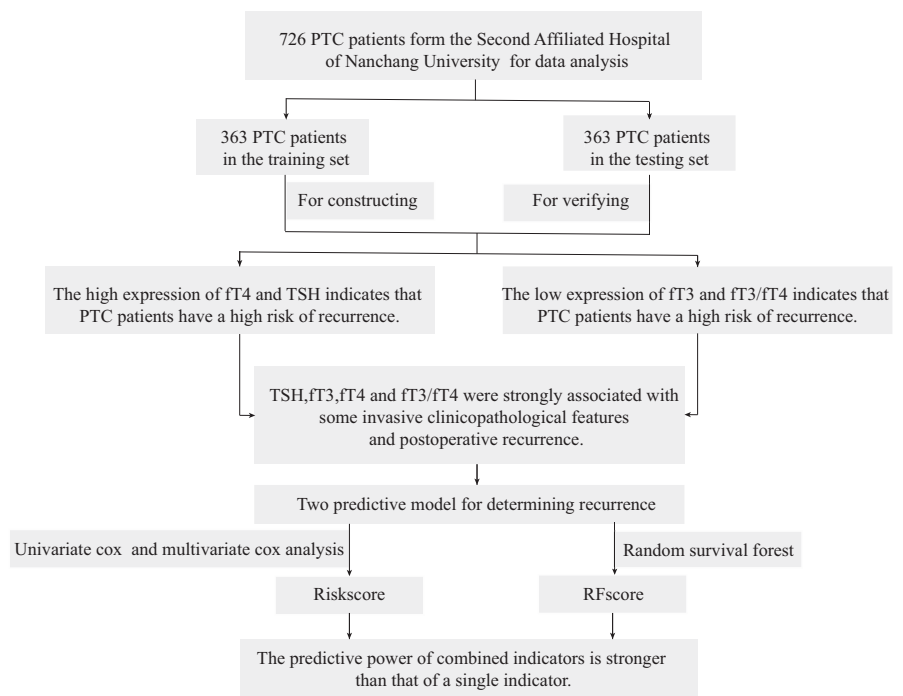# 2 | MATERIALS AND METHODS

2.1 | Patients

In this study, the data of 1578 patients with papillary thyroid carcinoma (PTC) treated at the Second Affiliated Hospital of Nanchang University from August 2018 to January 2022 were retrospectively reviewed; those with histologically confirmed PTC and complete preoperative laboratory data including preoperative serum TSH, free triiodothyronine (fT3), and free thyroxine (fT4) were included in the study. By contrast, patients with (1) other histological thyroid cancer types, such as medullary thyroid carcinoma, follicular thyroid carcinoma, and anaplastic thyroid cancer ($n = 361$); (2) with previous or coexisting malignant tumors ($n = 103$); (3) with other thyroid diseases, such as hyperthyroidism, hypothyroidism, and Hashimoto's thyroiditis($n = 236$); and (4) who used thyroid medications, such as Euthyrox ($n = 152$), were excluded. All patients signed an informed consent, and the study was approved by the ethics committee. Ultimately, 852 patients met the exclusion criteria, while the remaining 726 patients were included in our study and followed up by phone interview (Figure 1). Recurrent patients were defined as those with new masses found on any imaging examination and confirmed by pathological biopsy or surgery; disease-free survival (DFS) was defined as the period from the date of surgery to the date of recurrence diagnosis or last follow-up. The date of the last follow-up was February 21, 2021. Using the sample function in R, we randomly divided all patients ($n = 726$) into a training set ($n = 363$) and a testing set ($n = 363$) in a 1:1 ratio. All statistical models were fitted to the training set, while the testing set was used to judge the effect of the models.

2.2 | Data collection

The baseline data were obtained from the outpatient data. All laboratory data (blood chemistry analysis) were acquired from patients prior to surgery, and the tumor biopsy data were obtained from the patient's pathological and color Doppler ultrasound reports.

2.3 | Treatment

Based on the National Comprehensive Cancer Network guidelines, the standard treatment used in our study was thyroidectomy; patients' serum TSH, free triiodothyronine (fT3), and free thyroxine (fT4) levels were measured preoperatively. Blood samples were collected from each patient 8–10 h prior to surgery using an automatic chemiluminescence detection system (Cobas E411), which is commonly used for testing.

FIGURE 1 PTC patients exclusion flowchart

1578 PTC patients

(1) other histological thyroid cancers, such as medullary thyroid carcinoma, follicular thyroid carcinoma and anaplastic thyroid cancer (n=361)

1217 PTC patients

(2) previously or simultaneously malignant tumor (n=103)

1114 PTC patients

(3) other thyroid diseases such as hyperthyroidism, hypothyroidism and Hashimoto's thyroiditis (n=236)

878 PTC patients

(4) taking thyroid hormone drugs, such as Euthyrox (n=152)

726 PTC patients in final analysis

FIGURE 2 Data analysis process of the article

726 PTC patients form the Second Affiliated Hospital of Nanchang University for data analysis

363 PTC patients in the training set — For constructing

363 PTC patients in the testing set — For verifying

The high expression of fT4 and TSH indicates that PTC patients have a high risk of recurrence.

The low expression of fT3 and fT3/fT4 indicates that PTC patients have a high risk of recurrence.

TSH, fT3, fT4 and fT3/fT4 were strongly associated with some invasive clinicopathological features and postoperative recurrence.

Two predictive model for determining recurrence

Univariate cox and multivariate cox analysis → Riskscore

Random survival forest → RFscore

The predictive power of combined indicators is stronger than that of a single indicator.

2.4 | Statistical analysis

All statistical analyses were performed using the R software (3.6.1). Mann–Whitney *U*-test was used to analyze the difference between continuous variables, while chi-square test was used to analyze the difference between categorical variables. The receiver operating characteristic (ROC) curves were used to determine the optimal cut-off value of the variables, while the area under the curve (AUC) was used to reflect their predictive power. Univariate and multivariate Cox analyses were used to further analyze the predictive value of the variables. Random survival forest was used to build an integrated model based on decision trees, which could greatly improve the prediction performance. The Kaplan–Meier (K-M) curve was used to visualize the prognosis of the variables, while the log-rank test was

used to determine the corresponding *p*-value. A p-value of <0.05 was significant.

3 | RESULTS

3.1 | Clinical baseline characteristics

The data analysis process is shown in Figure 2. To predict the prognosis of PTC more accurately, a machine learning method was used to establish a model in order to predict recurrence. A total of 726 PTC patients were randomly divided into the training set and the testing set by a 1:1 ratio. The training set was used to construct the model, while the testing set was used to verify the predictive

effect of the model; the relationship between serum TSH, fT3, fT4, and fT3/fT4 prior to surgery and PTC recurrence was the focus of our study. The baseline characteristics included patient's age, sex, lymph node metastasis (LNM), unifocal or multifocal lesions, presence or absence of hypertension, maximum tumor diameter, immediate blood glucose level, LNM rate, TSH, fT3, fT4, and fT3/fT4. The Kolmogorov–Smirnov test was used to confirm the differences among the three groups (Table 1).

3.2 | Effects of TSH and thyroid hormone on PTC recurrence

To more accurately quantify the predictive ability of these four indicators, PTC recurrence was used as the endpoint, and "pROC" package[11] was used to construct the ROC curves of the training set. The results are shown in Figure 3A–D. The area under the curve (AUC) and 95% confidence intervals (95% CIs) of TSH, fT3, fT4, and fT3/fT4 were 0.682 (0.555–0.809, $p = 0.005$), 0.684 (0.565–0.804, $p = 0.002$), 0.649 (0.512–0.785, $p = 0.033$), and 0.736 (0.617–0.855, $p < 0.001$), respectively. The optimal cutoff values for these four indicators were 2.778 (specificity: 82.4%, sensitivity: 56.5%), 2.995 (specificity: 83.2%, sensitivity: 47.8%), 1.405 (specificity: 79.7%,

sensitivity: 52.2%), and 2.439 (specificity: 74.7%, sensitivity: 69.6%). According to the optimal cutoff values, the patients in the training set were divided into high and low groups to determine whether the concentrations of TSH, fT3, fT4, and fT3/fT4 were correlated with the recurrence of PTC; the ROC curves of the testing set (Figure 4A–D) and the total set (Figure 4E–H) were also constructed, which showed that the four indicators have good predictive ability.

The AUC of fT3/fT4 was the largest, suggesting that fT3/fT4 had a strong ability to predict PTC recurrence. Previous studies have shown that TSH can be used as one of the risk factors for PTC recurrence[12,13], but the association between fT3, fT4, or fT3/fT4 as a single indicator and the recurrence of PTC has not been reported. In TN breast cancer, fT3 may be involved in the transduction of proliferation signals[14]. Strzałka A et al. found that fT3 also contributes to the development of pancreatic cancer[15]. Therefore, the predictive effect of fT3 on the recurrence of PTC should be explored further. Aron Margaret et al. evaluated the association between ablative fT4 to thyroglobulin (TG) ratio and recurrence in DTC patients, and found that an fT4/TG ratio of <27% could be used as a predictor of recurrence[16]. Therefore, it is reasonable to infer that fT3, fT4, and fT3/fT4 may have a certain correlation with the recurrence of PTC. The results of our study showed that fT3/fT4 was an ideal predictor of recurrence. It might become one of the clinical research directions of PTC recurrence in the future.

TABLE 1 Baseline characteristics of PTC patients

Characteristics	Training set	Testing set	Total set	p
	N/Mean ± SD			
Number of patients	n = 363	n = 363	n = 726	–
Age	46.99 ± 12.92	46.12 ± 13.52	46.56 ± 13.22	0.595
Gender				
Male	80(22.04%)	71(19.56%)	151(20.80%)	0.713
Female	283(77.96%)	292(80.44%)	575(79.20%)	
LNM				
Yes	125(34.44%)	130(35.81%)	255(35.12%)	0.927
No	238(65.56%)	233(64.19%)	471(64.88%)	
Lesions				
Unifocal	273(75.21%)	267(73.55%)	540(74.38%)	0.878
Multifocal	90(24.79%)	96(26.45%)	186(25.62%)	
Hypertension				
Yes	207(57.02%)	190(52.34%)	397(54.68%)	0.448
No	156(42.98%)	173(47.66%)	329(45.32%)	
Maximum tumor diameter(cm)	1.18 ± 0.84	1.25 ± 0.90	1.21 ± 0.87	0.410
Glucose	6.23 ± 1.66	6.14 ± 1.66	6.18 ± 1.66	0.492
LNM rate	0.18 ± 0.30	0.16 ± 0.27	0.17 ± 0.29	0.999
TSH	1.89 ± 1.14	1.80 ± 1.07	1.84 ± 1.11	0.711
fT3	3.38 ± 0.59	3.36 ± 0.60	3.37 ± 0.60	0.499
fT4	1.26 ± 0.21	1.25 ± 0.20	1.26 ± 0.21	0.814
fT3/fT4	2.75 ± 0.63	2.73 ± 0.56	2.74 ± 0.60	0.797

Abbreviations: fT3, free triiodothyronine; fT4, free thyroxine; LNM, lymph node metastasis; PTC, papillary thyroid cancer; TSH, thyrotrophin.

In order to further investigate the association between the expression of each indicator and the recurrence of PTC, a K-M analysis of the disease-free survival (DFS) of all four indicators was performed, which were grouped according to the optimal cutoff values using "survival" package[17] (Figure 5A–D). PTC patients with higher TSH ($p < 0.001$) and fT4 ($p = 0.002$) levels had a higher risk of PTC recurrence; those with lower fT3 ($p = 0.002$) and fT3/fT4 ($p < 0.001$) levels also had a higher risk of PTC recurrence. Based on their optimal cutoff values of the training set, the same method was applied to the testing set (Figure 6A–D) and the total set (Figure 6E–H). By conducting two validations in the testing set and the total set, it was confirmed that the grouping of the four indicators in the training set has certain repeatability and accuracy; however, more clinical data are still required to support our results.

High TSH expression is one of the risk factors for PTC recurrence[12,13]. The study by Benjamin et al. showed that in patients with primary breast cancer, increase in fT4 levels and decrease in fT3/fT4 levels could be regarded as risk factors for cancer recurrence[9]. This conclusion is consistent with the results of our study. fT3/fT4 has been proven to be the major prognostic marker in advanced metastatic

colorectal cancer[18]; our study also showed that fT3/fT4 is a good predictor of PTC recurrence, suggesting its clinical application value.

3.3 | Clinical correlation test

In addition, the association of clinical baseline characteristics with TSH, fT3, fT4, and fT3/fT4 was investigated (Table 2). In our study, significant differences were observed in the gender ($p = 0.004$), maximum tumor diameter ($p = 0.002$), LNM ($p < 0.001$), multifocal lesions ($p = 0.002$), LNM rate ($p < 0.001$), and fT3 ($p < 0.001$) between the high and low TSH groups. Significant differences were also found in the age ($p = 0.029$), gender ($p < 0.001$), LNM ($p = 0.013$), LNM rate ($p = 0.021$), TSH ($p = 0.015$), fT4 ($p = 0.005$), and fT3/fT4 ($p < 0.001$) between the high and low fT3 groups. Moreover, significant differences were observed in the LNM ($p < 0.001$), LNM rate ($p < 0.001$), TSH ($p = 0.016$), fT3 ($p < 0.001$), and fT3/fT4 ($p < 0.001$) between the high and low fT4 groups. Furthermore, significant differences were observed in the age ($p = 0.002$), gender ($p = 0.028$), maximum tumor diameter ($p = 0.017$), LNM ($p = 0.003$), multifocal lesions ($p = 0.049$),
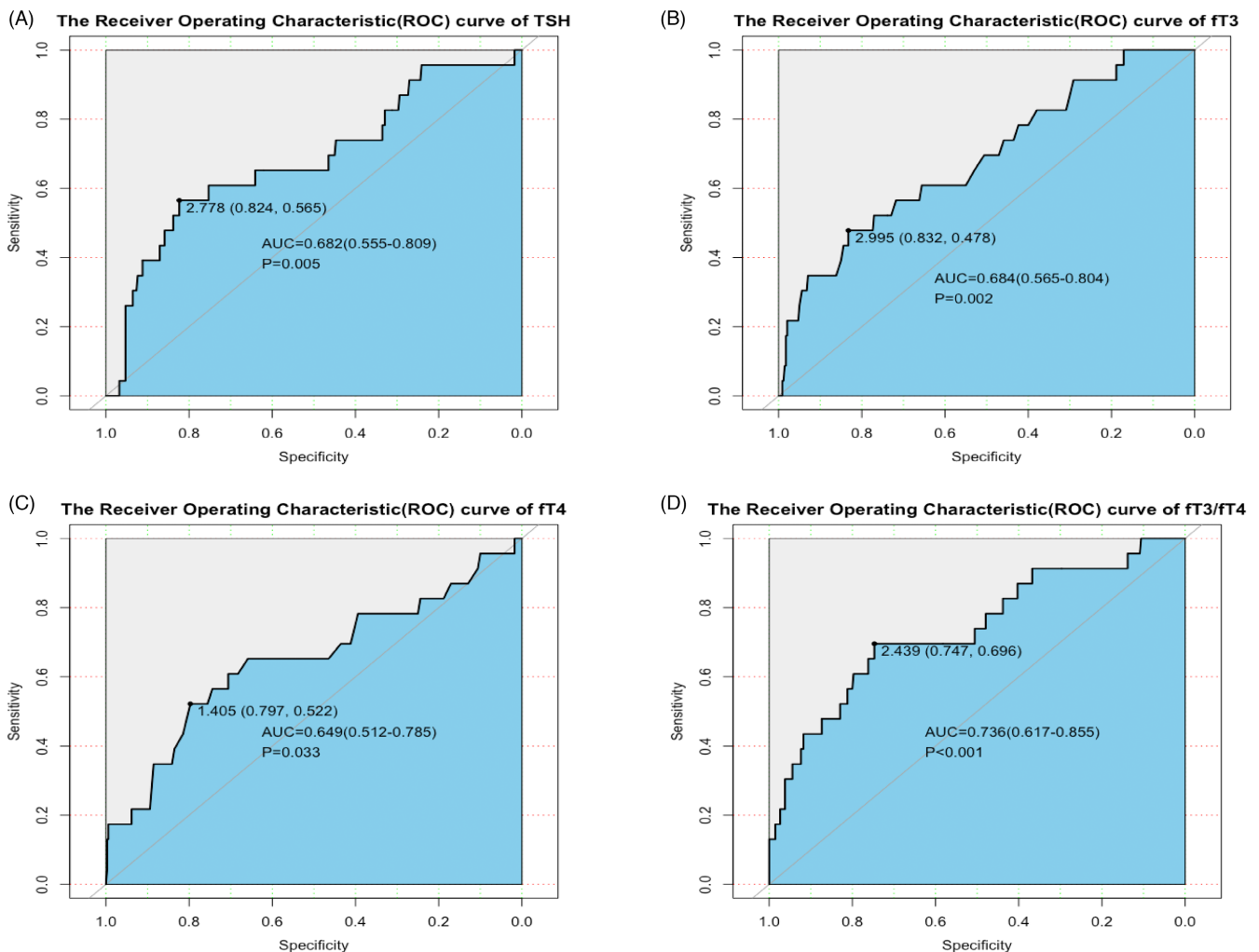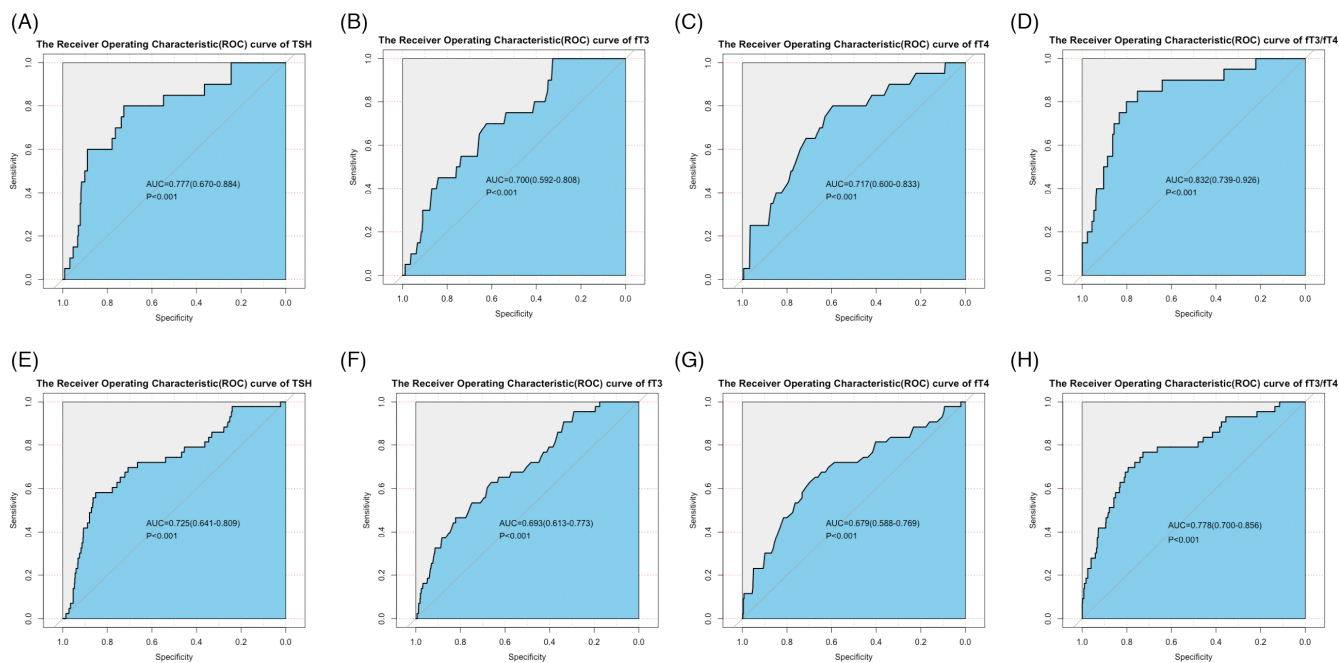

FIGURE 3 Receiver operating characteristics(ROC) curve of TSH (A),fT3 (B),fT4 (C), and fT3/fT4 (D) for disease-free survival(DFS) status among 363 patients with PTC in training set

FIGURE 4 Receiver operating characteristics(ROC) curve of TSH (A),fT3 (B),fT4 (C), and fT3/fT4 (D) for disease-free survival(DFS) status in testing set and that of TSH (E),fT3 (F),fT4 (G), and fT3/fT4 (H) for disease-free survival(DFS) status in total set

immediate blood glucose level ($p = 0.020$), LNM rate ($p = 0.007$), fT3 ($p < 0.001$), and fT4 ($p < 0.001$) between the high and low fT3/fT4 groups. The same clinical data analysis method was also adopted for the training set and the testing set. All results are shown in Table S1 and Table S2. A comprehensive analysis of the three tables was performed; results showed that age, gender, maximum tumor diameter, LNM, multifocal lesions, hypertension, immediate blood glucose level, and LNM rate may have potential relationships with TSH, fT3, fT4, and fT3/fT4. In addition to comparing the baseline characteristics, the correlation between these four indicators was also analyzed, and a certain correlation was found among TSH, fT3, and fT4, while no correlation was found between TSH and fT3/fT4. What caught our attention was the extremely close correlation between TSH and fT3, which requires an in-depth investigation and may be related to the signal transduction pathway of PI3K[19].

Aa et al. found that the serum TSH level was higher in patients with LNM, while the serum TSH level in patients with aggressive PTC was higher than that in non-aggressive patients[20]. This finding indicates that the higher the degree of the tumor malignancy, the higher the serum TSH level, thus increasing the risk of PTC recurrence. Our study also demonstrated that high levels of TSH can be a risk factor for PTC recurrence. Fitzgerald Stephen P et al. examined the association between clinical parameters and thyroid hormone levels and TSH levels, and found that the thyroid hormone levels seemed to have a stronger correlation with the clinical parameters compared with the TSH levels. The correlation between clinical parameters and TSH levels can be explained by the strong negative correlation between thyroid hormone and TSH[21]. Although the clinical parameters included in the study varied, it should be investigated whether the clinical and research portion of current thyroidology

should be based on the reference TSH levels in order to determine the thyroid status.

3.4 | Three models for predicting recurrence

In order to verify our speculation, recurrence was assigned as the endpoint, and a univariate Cox analysis[22] of all indicators was performed (Table 3). Only maximum tumor diameter (HR: 4.098, 95% CI: 1.605–10.470, $p = 0.003$), LNM (HR: 4.366, 95% CI: 1.790–10.650, $p = 0.001$), multifocal lesions (HR: 3.078, 95% CI: 1.357–6.981, $p = 0.007$), TSH (HR: 6.007, 95% CI: 2.567–14.060, $p < 0.001$), fT3 (HR: 3.452, 95% CI: 1.515–7.862, $p = 0.003$), fT4 (HR: 3.433, 95% CI: 1.491–7.904, $p = 0.004$), and fT3/fT4 (HR: 5.110, 95% CI: 2.085–12.520, $p < 0.001$) were significant. Therefore, these seven indicators were included in our subsequent analysis.

Considering that there may be an internal correlation among them, the correlation between these seven indicators and recurrence was further analyzed using the COX-PH algorithm[23], and two models were established for predicting recurrence according to whether recursive elimination was applied (Table 4). In the model in which recursive elimination was not applied (multivariable Cox 1), maximum tumor diameter (HR: 2.763, 95% CI: 1.039–7.345, $p = 0.042$), LNM (HR: 2.627, 95% CI: 1.045–6.607, $p = 0.040$), TSH (HR: 4.540, 95% CI: 1.888–10.918, $p < 0.001$), and fT3/fT4 (HR: 3.439, 95% CI: 1.009–11.723, $p = 0.048$) were significant; TSH and fT3/fT4 had the highest contribution rates, with coefficients of 1.513 and −1.235, respectively. After using recursive elimination (multivariable Cox 2), the maximum tumor diameter (HR: 2.907, 95% CI: 1.119–7.554, $p = 0.028$), LNM (HR:
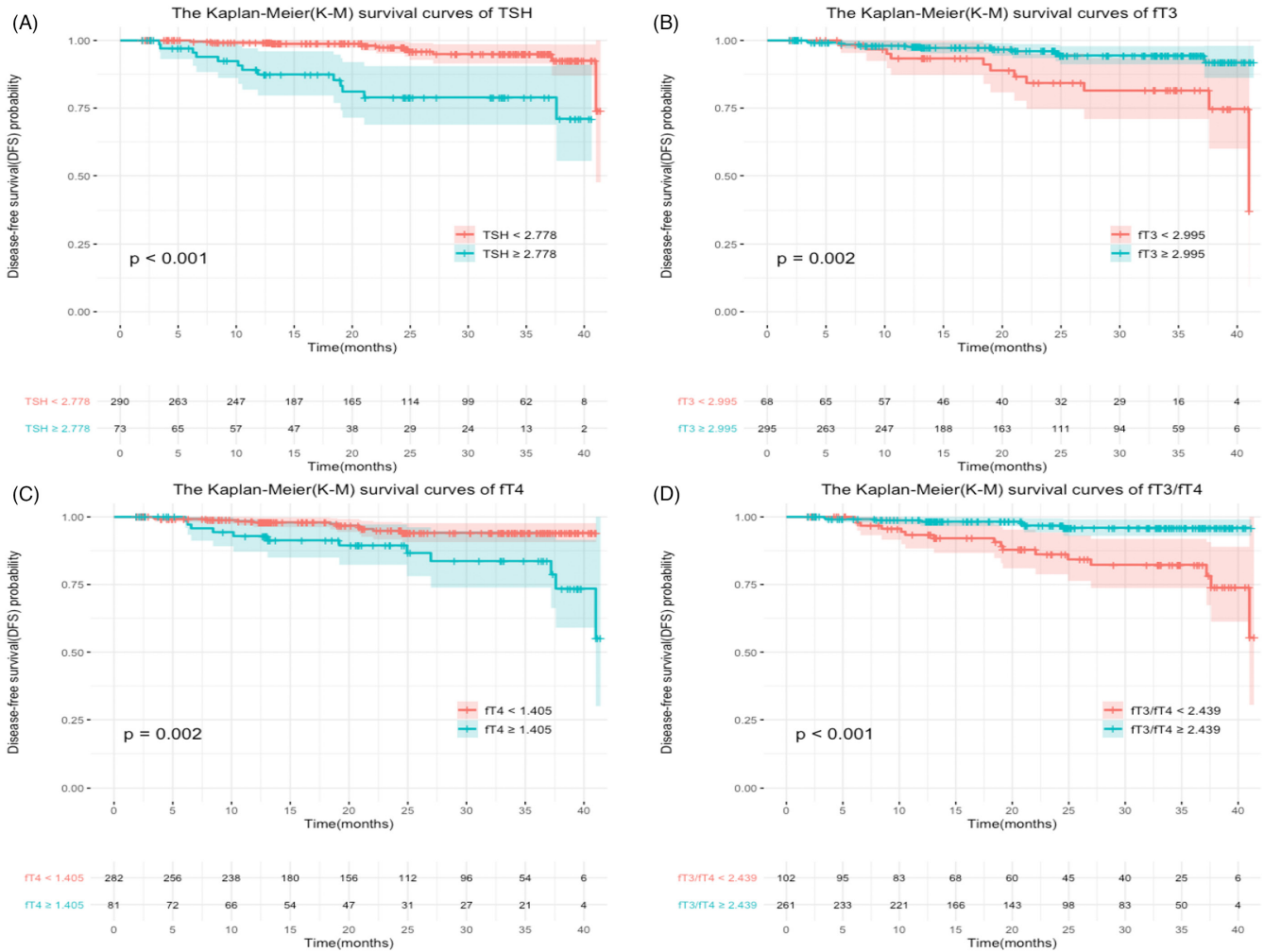
FIGURE 5 Correlation between the level of four indicators and PTC recurrence in training set. (A) TSH≥2.778 was associated with poor DFS rate ($p < 0.001$). (B) fT3 < 2.995 was associated with poor DFS rate ($p < 0.002$). (C) fT4 ≥ 1.405 was associated with poor DFS rate ($p = 0.002$). (D) fT3/fT4 < 2.439 was associated with poor DFS rate ($p < 0.001$)
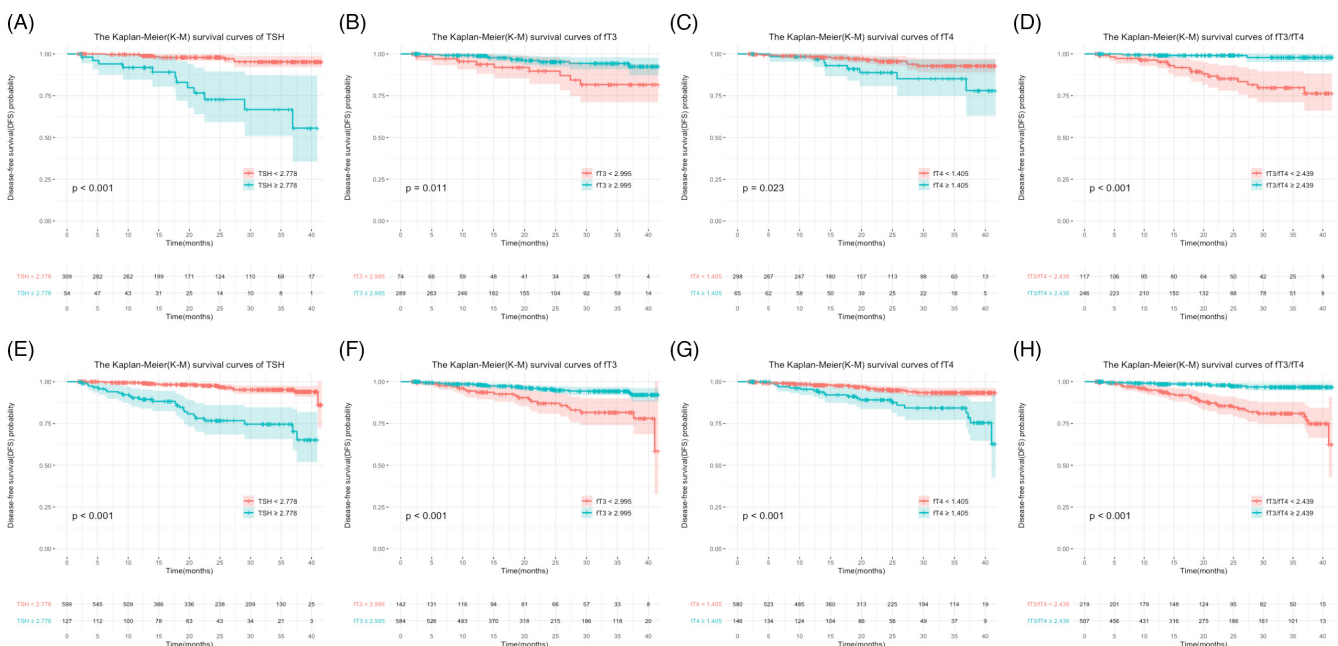
FIGURE 6 Correlation between the level of four indicators and PTC recurrence in testing set (A–D) and total set (E–H)

TABLE 2 Correlation between four indicators and clinicopathological characteristics of PTC patients in total set

Characteristics	TSH <2.778 (n = 599)	TSH ≥2.778 (n = 127)	P	fT3 <2.995 (n = 142)	fT3 ≥2.995 (n = 584)	P	fT4 <1405 (n = 580)	fT4 ≥1405 (n = 146)	P	fT3/fT4 <2.439 (n = 219)	fT3/fT4 ≥2.439 (n = 507)	p
Age(years)#	46.3(13.0)	47.9(14.3)	0.322	48.8(14.0)	46.0(13.0)	0.029*	46.8(13.3)	45.5(13.0)	0.282	49.0(13.2)	45.5(13.1)	0.002*
Gender												
Male	137(22.9%)	14(11.0%)	0.004*	14(9.9%)	137(23.5%)	<0.001*	115(19.8%)	36(24.7%)	0.242	34(15.5%)	117(23.1%)	0.028*
Female	462(77.1%)	113(89.0%)		128(90.1%)	447(76.5%)		465(80.2%)	110(75.3%)		185(84.5%)	390(76.9%)	
Maximum tumor diameter(cm)												
≤1 cm	336(56.1%)	51(40.2%)	0.002*	78(54.9%)	309(52.9%)	0.735	309(53.3%)	78(53.4%)	0.999	132(60.3%)	255(50.3%)	0.017*
>1 cm	263(43.9%)	76(59.8%)		64(45.1%)	275(47.1%)		271(46.7%)	68(46.6%)		87(39.7%)	252(49.7%)	
LNM												
No	409(68.3%)	62(48.8%)	<0.001*	79(55.6%)	392(67.1%)	0.013*	395(68.1%)	76(52.1%)	<0.001*	124(56.6%)	347(68.4%)	0.003
Yes	190(31.7%)	65(51.2%)		63(44.4%)	192(32.9%)		185(31.9%)	70(47.9%)		95(43.4%)	160(31.6%)	
Lesions												
Unifocal	460(76.8%)	80(63.0%)	0.002*	104(73.2%)	436(74.7%)	0.810	435(75.0%)	105(71.9%)	0.512	174(79.5%)	366(72.2%)	0.049*
Multifocal	139(23.2%)	47(37.0%)		38(26.8%)	148(25.3%)		145(25.0%)	41(28.1%)		45(20.5%)	141(27.8%)	
Hypertension												
Yes	328(54.8%)	69(54.3%)	0.999	77(54.2%)	320(54.8%)	0.978	315(54.3%)	82(56.2%)	0.757	121(55.3%)	276(54.4%)	0.904
No	271(45.2%)	58(45.7%)		65(45.8%)	264(45.2%)		265(45.7%)	64(43.8%)		98(44.7%)	231(45.6%)	
Glucose#	6.14(1.52)	6.40(2.18)	0.787	6.48(1.94)	6.11(1.58)	0.154	6.15(1.62)	6.29(1.81)	0.643	6.45(1.81)	6.06(1.57)	0.020*
LNM rate#	0.16(0.28)	0.23(0.30)	<0.001*	0.20(0.29)	0.16(0.28)	0.021*	0.15(0.28)	0.24(0.32)	<0.001*	0.20(0.30)	0.16(0.28)	0.007*
TSH#	-	-		2.04(1.11)	1.80(1.11)	0.015*	1.89(1.11)	1.67(1.11)	0.016*	1.84(1.04)	1.85(1.14)	0.975
fT3#	3.40(0.61)	3.22(0.48)	<0.001*	-	-		3.33(0.55)	3.54(0.73)	<0.001*	3.00(0.34)	3.53(0.61)	<0.001*
fT4#	1.25(0.20)	1.26(0.23)	0.821	1.22(0.23)	1.26(0.20)	0.005*	-	-		1.40(0.19)	1.19(0.18)	<0.001*
fT3/fT4#	2.77(0.61)	2.62(0.49)	0.093	2.32(0.44)	2.84(0.58)	<0.001*	2.85(0.57)	2.30(0.46)	<0.001*	-	-	-

#Mean(standard deviation).
*p<0.05 considered as statistically significant.

TABLE 3 Univariate Cox proportional hazards regression analysis for disease-free survival(DFS) in PTC patients

Characteristics	Univariate Cox	
	HR(95%CI)	p
Age		0.801
<45	Reference	
≥45	1.112(0.486–2.544)	
Gender		0.102
Male	Reference	
Female	0.297(0.069–1.271)	
Hypertension		0.457
No	Reference	
Yes	0.733(0.323–1.662)	
Maximum tumor diameter(cm)		0.003*
≤1 cm	Reference	
>1 cm	4.098(1.605–10.470)	
LNM		0.001*
No	Reference	
Yes	4.366(1.790–10.650)	
Lesions		0.007*
Unifocal	Reference	
Multifocal	3.078(1.357–6.981)	
TSH		<0.001*
<2.778	Reference	
≥2.778	6.007(2.567–14.060)	
fT3		0.003*
≥2.995	Reference	
<2.995	3.452(1.515–7.862)	
fT4		0.004*
<1.405	Reference	
≥1.405	3.433(1.491–7.904)	
fT3/fT4		<0.001*
≥2.439	Reference	
<2.439	5.110(2.085–12.520)	

Abbreviations: 95% CI, 95% confidence interval; HR, hazard ratio; LNM: lymph node metastasis; PTC, papillary thyroid carcinoma.

*$p < 0.05$ considered as statistically significant.

2.792, 95% CI: 1.124–6.932, $p = 0.027$), TSH (HR: 4.556, 95% CI: 1.904–10.902, $p < 0.001$), and fT3/fT4 (HR: 4.570, 95% CI: 1.849–11.299, $p = 0.001$) were significant. TSH and fT3/fT4 still had the largest contribution rates. This finding suggests that TSH and fT3/fT4 may have a strong clinical application value. By comparing the AIC and C-index of the two models, we found that multivariable Cox 2 (AIC = 203.05, C-index = 0.85) is more accurate than multivariable Cox 1 (AIC = 206.33, C-index = 0.85).

Next, the risk score of each patient was defined according to the multivariable Cox 2:

$$Risk\ scor = \sum_{i=1}^{n} Characteristics \times Coef$$

where n is the number of characteristics, characteristics are the binary clinical characteristics in the signature, and Coef is the estimated regression coefficient value from the Cox-PH algorithm.

Finally, the risk score was calculated as follows: maximum tumor diameter $\times 1.067 + $ LNM $\times 1.027 + $ TSH $\times 1.517 + $ fT3/fT4 $\times -1.520$.

Using the risk score as the standard value, an ROC curve (Figure 7A) and a K-M curve (Figure 7B) were also constructed for the training set, which indicated that its prediction ability was excellent.

To determine whether a difference exist between the predictive power of risk score and the four indicators, the AUC between risk score and the four indicators were compared; the results are shown in Figure 7C. Among them, the risk score has the highest AUC and the strongest predictive ability. A significant difference was observed between the risk score and TSH ($p < 0.001$), fT3 ($p < 0.001$), fT4 ($p = 0.005$), and fT3/fT4 ($p = 0.022$), which further indicated that the risk score had the best predictive ability.

Moreover, the random survival forest[24] was used to build the RF score for analyzing the seven indicators; TSH and fT3/fT4 still had the highest contribution rate (Figure 8A–C). The same approach was also applied to both the testing set (Figure 9A,B,E,F) and total set (Figure 9C,D,G,H) to verify the effect of the model.

Finally, the predictive ability of each indicator was analyzed and compared; the combined indicators were found to have a better predictive ability than the single indicator. When the AUC of the three models was compared, the RF score had the best predictive power among them. TSH and fT3/fT4 were always the indicators with the highest contribution rate. This finding reflects the advanced point of this study. In clinical practice, the PTC recurrence prediction model constructed in this study is indeed operable to a certain extent, but more experimental data are needed to support its accuracy.

4 | DISCUSSION

In this study, the association of TSH, fT3, fT4, and fT3/fT4 with PTC recurrence was investigated. Most of the previous studies evaluating the risk of recurrence in PTC patients have focused on a single indicator and clinical parameters; this study was the first to use machine learning methods to comprehensively analyze TSH, fT3, fT4, and fT3/fT4, and to explore the ability of a combination of indicators to judge the risk of PTC recurrence. Combining the four indicators significantly improved the ability to predict recurrence in patients; therefore, the internal association among these indicators and how they increase the risk of recurrence warrants further exploration.

Current studies have shown that TSH can regulate the production of T3 and T4.TSH is a hormone secreted by the adenohypophysis. It stimulates the secretion of thyrotropin-releasing hormone secreted by the hypothalamus. The production of TRH inhibited by the thyroid hormone negative feedback[25]. Thyroid hormone synthesis, initiated by the intake of iodine, is primarily regulated by the binding of TSH to its homologous receptor (TSHR). When activated

TABLE 4 Multivariable Cox proportional hazards regression analysis for disease-free survival(DFS) in PTC patients

Characteristics	Multivariable Cox 1			Multivariable Cox 2		
	Coef	HR(95%CI)	p	Coef	HR(95%CI)	p
Maximum tumor diameter(cm)	1.016		0.042*	1.067		0.028*
≤1 cm		Reference			Reference	
>1 cm		2.763(1.039–7.345)			2.907(1.119–7.554)	
LNM	0.966		0.040*	1.027		0.027*
No		Reference			Reference	
Yes		2.627(1.045–6.607)			2.792(1.124–6.932)	
Lesions	0.738		0.092	0.723		0.102
Unifocal		Reference			Reference	
Multifocal		2.092(0.886–4.940)			2.060(0.867–4.894)	
TSH	1.513		<0.001*	1.517		<0.001*
<2.778		Reference			Reference	
≥2.778		4.540(1.888–10.918)			4.556(1.904–10.902)	
fT3	−0.161		0.761			
≥2.995		Reference				
<2.995		1.174(0.416–3.315)				
fT4	0.406		0.401			
<1.405		Reference				
≥1.405		1.500(0.582–3.870)				
fT3/fT4	−1.235		0.048*	−1.520		0.001*
≥2.439		Reference			Reference	
<2.439		3.439(1.009–11.723)			4.570(1.849–11.299)	
Model effect	AIC = 206.33 C-index = 0.85			AIC = 203.05 C-index = 0.85		

Abbreviations: 95% CI, 95% confidence interval; AGR, albumin/globulin ratio; HR, hazard ratio; LMR; lymphocyte/monocyte ratio; LNM, lymph node metastasis; NLR, neutrophil/lymphocyte ratio; PLR, platelet/lymphocyte ratio; PTC, papillary thyroid carcinoma.
*$p < 0.05$ considered as statistically significant.

by iodide, the TSHR is transported into the thyroid cells by sodium iodide symbiosis and is oxidized by thyroid peroxidase (TPO). Excessive production and/or lack of hydrogen peroxide degradation may contribute to the development of inflammatory and neoplastic diseases in the thyroid[26]. Thyroglobulin (TG) is synthesized by iodization, which is catalyzed by TPO, and then, the coupling reaction is carried out to form T4 or T3[27].
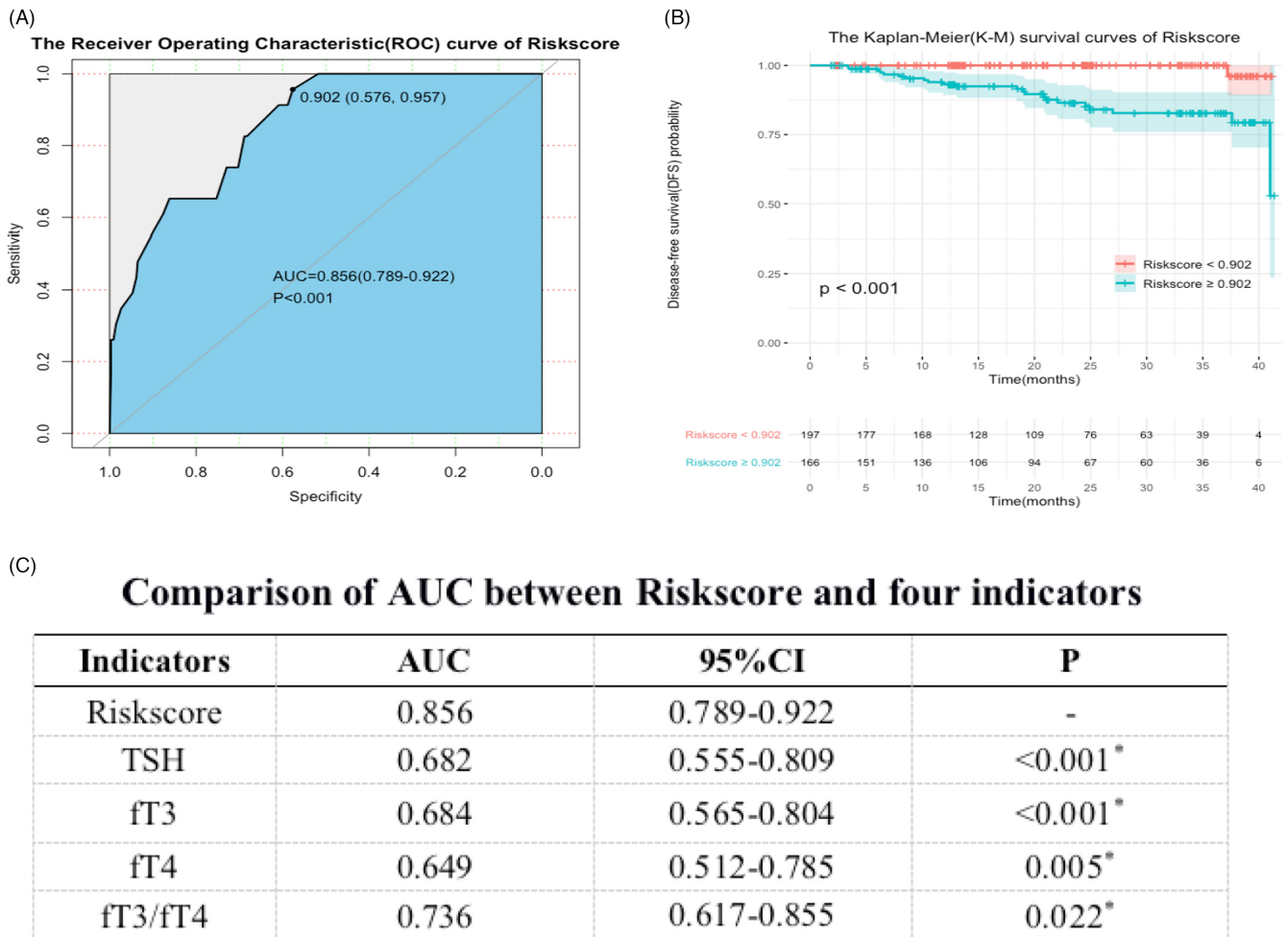
Considering that the four of them are intrinsically related, it is possible that they contribute to the recurrence of PTC. Hence, we attempted to explore the exact mechanism that leads to PTC recurrence.

The TSH signal is transmitted in several pathways, and each pathway has internal cross-connection. Protein kinase A (PKA) may be one of the key junctions of TSH and thyroid hormone affecting recurrence. The binding of TSH to TSHR leads to the coupling of Gsα, which in turn activates the adenylate cyclase to form cAMP,

causes phosphorylation of PKA, and induces the activation of downstream proteins in the cytoplasm and nucleus. This cascade is a major regulator of thyroid hormone synthesis, growth, and differentiation. TSH-induced cell proliferation in PTC can be dependent on the TSHR/cAMP/PKA/PAK4 signaling. TSH induces the increase in PAK4 activity, and PAK4 can inhibit cell adhesion and promote cell proliferation and the invasion of thyroid cancer cells[28].

In addition, adipocytes and insulin regulation may also play an important role in PTC recurrence.

Metabolic syndrome (METS) comprises several common nutritional metabolic disorders, presenting a phenomenon of symptom aggregation[29]. Insulin resistance (IR) is recognized as the main link in the pathogenesis of METS and one of the primary mechanisms by which METS affects the occurrence and development of malignant tumors[30]. Park et al. evaluated the association between METS and thyroid cancer, and showed that METS was associated with an

(A)

The Receiver Operating Characteristic(ROC) curve of Riskscore

0.902 (0.576, 0.957)

AUC=0.856(0.789-0.922)
P<0.001

(B)

The Kaplan-Meier(K-M) survival curves of Riskscore

p < 0.001

Riskscore < 0.902
Riskscore ≥ 0.902

Riskscore < 0.902	197	177	168	128	109	76	63	39	4
Riskscore ≥ 0.902	166	151	136	106	94	67	60	36	6
	0	5	10	15	20	25	30	35	40

Time(months)

(C)

Comparison of AUC between Riskscore and four indicators

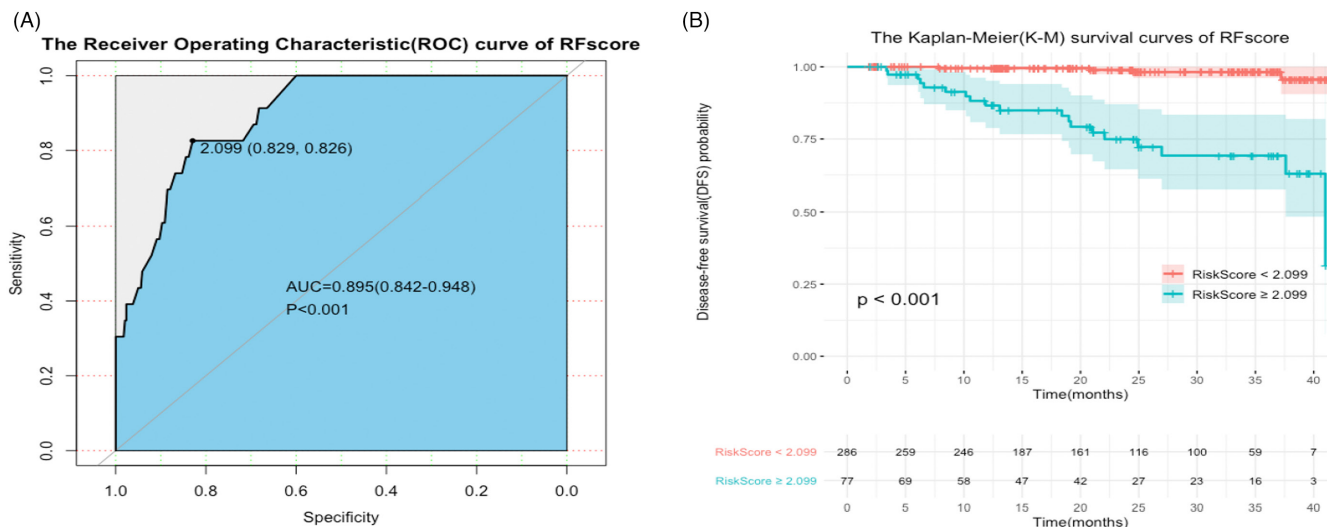| Indicators	AUC	95%CI	P
Riskscore	0.856	0.789-0.922	-
TSH	0.682	0.555-0.809	<0.001*
fT3	0.684	0.565-0.804	<0.001*
fT4	0.649	0.512-0.785	0.005*
fT3/fT4	0.736	0.617-0.855	0.022*

FIGURE 7 Predictive power of risk score on patient DFS status and DFS rate. (A) The receiver operating characteristics(ROC) curve of risk score. (B) risk score ≥0.902 was associated with poor DFS rate ($p < 0.001$). (C) Comparison of the area under the ROC curves among risk score and four indicators for prognosis of PTC patients

increased risk of thyroid cancer[31]. The development of METS is positively correlated with TSH[32], and low fT4 level is an independent risk factor for METS[33]. TSH receptors are present in several cell types, including adipocytes[34]. TSH binds to the receptors of adipocytes and stimulates the production of IL-6, which then mediates the secretion of leptin[35]. T3 can use the PI3K signaling pathway to upregulate the expression of leptin in adipocytes, while ectopic fat plays an important role in the development of IR[36]. Therefore, adipocytes may be one of the key factors in the association between TSH, fT3, fT4, and IR. Moreover, PI3K/Akt is one of the key signaling pathways by which iodine and SPANXA1 promote the development of thyroid cancer[37]. Interestingly, PI3K can be activated by G-protein-coupled receptors (such as TSHR) and tyrosine kinase receptors (such as insulin receptor IR). Therefore, TSH and IR synergistically induced thyroid cell proliferation.

Considering the association between TSH and thyroid hormones and their influence on the recurrence of PTC through the related junctions such as PKA, adipocytes, and insulin regulation, we reasonably believe that the combination of these characteristics in the prediction of recurrence can improve the prediction efficiency to a certain extent, which is consistent with our results.

Our results showed that patients with high preoperative serum TSH and fT4 levels have high risk of PTC recurrence, while those with high levels of fT3 and fT3/fT4 have low risk of PTC recurrence. Previous studies have shown that high TSH level may be an independent predictor of PTC recurrence[12,13], while the role of fT3/fT4 in predicting PTC recurrence has not been reported. Some studies have shown that serum fT3 level is negatively correlated with the inflammatory state[38], and inflammation is also intrinsically correlated with thyroid cancer, suggesting that the relationship between fT3, inflammatory state, and thyroid cancer may require further discussion. The levels of fT4 play different roles in different cancer types. In liver cancer, a decrease in fT4 levels suggests an increased risk of death[39]; in primary breast cancer patients, an increase in fT4 levels is associated with a poor prognosis. In this study, high levels of fT4 in papillary thyroid cancer patients increase the risk of recurrence, which may be related to METS. Although TSH is currently recognized as the best indicator of thyroid function in clinical practice, other analyses show that thyroid hormone levels, especially fT4, seem to be more strongly correlated with clinical parameters compared with TSH levels[21]. This finding is different from the results of our study and suggests that the role of fT4 in cancer needs a

(A)

The Receiver Operating Characteristic(ROC) curve of RFscore

2.099 (0.829, 0.826)

AUC=0.895(0.842-0.948)
P<0.001

Specificity · Sensitivity

(B)

The Kaplan-Meier(K-M) survival curves of RFscore

p < 0.001

RiskScore < 2.099
RiskScore ≥ 2.099

Disease-free survival(DFS) probability · Time(months)

	0	5	10	15	20	25	30	35	40
RiskScore < 2.099	286	259	246	187	161	116	100	59	7
RiskScore ≥ 2.099	77	69	58	47	42	27	23	16	3

(C)

Comparison of AUC between RFscore and four indicators

Indicators	AUC	95%CI	P
RFscore	0.895	0.842-0.948	-
TSH	0.682	0.555-0.809	<0.001*
fT3	0.684	0.565-0.804	<0.001*
fT4	0.649	0.512-0.785	<0.001*
fT3/fT4	0.736	0.617-0.855	0.003*

FIGURE 8 The predictive power of RF score on patient DFS status and DFS rate. (A) The receiver operating characteristics(ROC) curve of RFscore. (B) RF score ≥2.099 was associated with poor DFS rate ($p < 0.001$). (C) Comparison of the area under the ROC curves among RF score and four indicators for prognosis of PTC patients

more in-depth analysis. As an important biochemical indicator, the level of fT3/fT4 is also the focus of research. A reduction in fT3/fT4 levels is associated with poor prognosis of primary breast cancer[9] and advanced metastatic colorectal cancer[18]; however, its association with PTC recurrence has not yet been reported. The effect of fT3/fT4 on the recurrence of PTC patients was analyzed, and it was found for the first time that fT3/fT4 can be a good predictor of PTC recurrence.

In addition to analyzing the correlation between a single indicator and PTC recurrence, a machine learning method was also used to establish three models for predicting PTC recurrence. The results showed that the predictive power of a combination of indicators was significantly stronger than that of a single indicator, which reflects the advancement of this study. Moreover, TSH and fT3/fT4 contributed the most to the model among all the indicators, suggesting that the internal correlation between these indicators and their new directions for clinical application should be explored further.

Although the predictive effect of our model is ideal, our study still lacks external data for verification. Our current study used limited data, and its conclusions may be influenced by the data selected. Hence, more data should be obtained for long-term follow-up to prove the accuracy of the model. In general, the association between TSH and thyroid hormones as well as their common influence on PTC recurrence are worthy of further investigation.

5 | CONCLUSION

Our results suggest that fT3/fT4 and TSH have a good ability to predict PTC recurrence. A combination of indicators is a better predictor of postoperative recurrence. The predictive power of the RF score established in this study is better than that of the risk score. However, more samples are needed to further validate our findings.
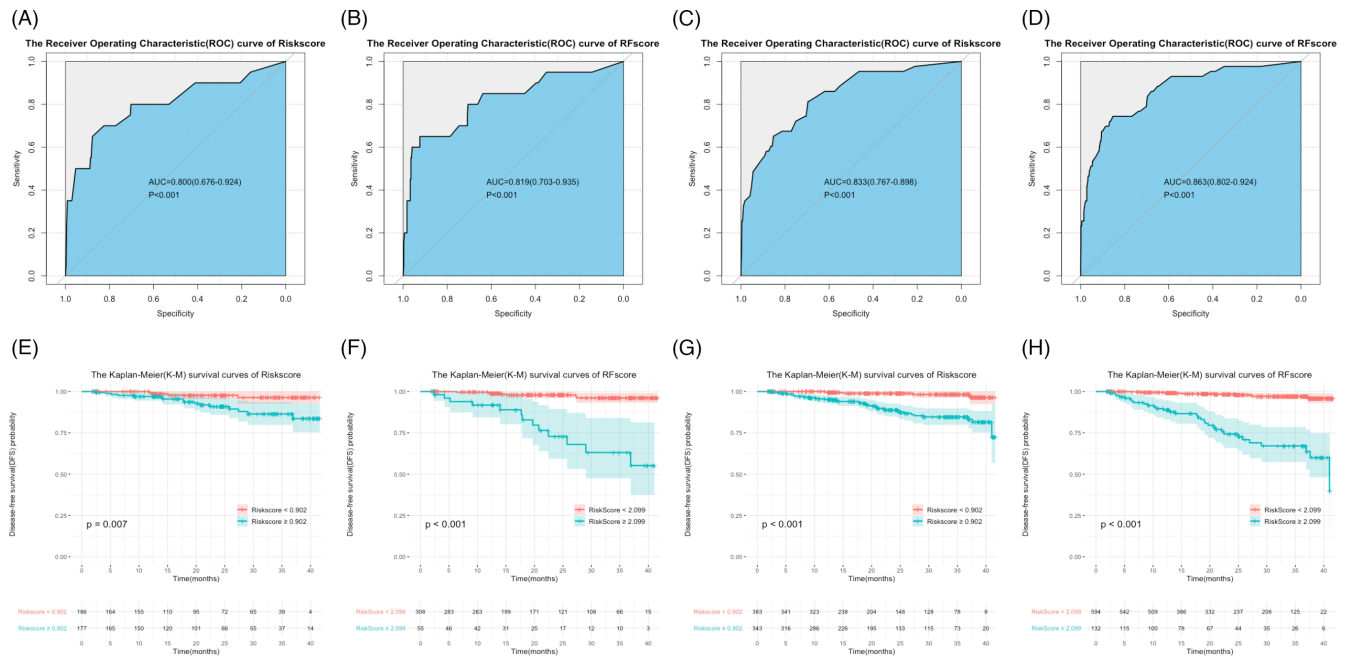
FIGURE 9 Receiver operating characteristics(ROC) curve of risk score (A) and RF score (B) in testing set and that of risk score (C) and RF score (D) in total set. Correlation between PTC recurrence and the level of risk score in testing set (E) and total set (G) and that between PTC recurrence and the level of RF score in testing set(F) and total set(H)

AUTHOR CONTRIBUTIONS

JCY, YXL, YSL, YYH, GHM, TZ, QH, and CQS jointly designed this study. JCY, YXL, LYS, and YYH collected clinical data from PTC patients. JCY, YXL, GHM, TZ, and QH further collated and preliminarily analyzed the data. JCY and YSL conducted statistical analysis and drew the figures and tables of the whole article. YXL, YSL, and YYH wrote the results section of the article, while GHM wrote the rest of the article. YYH, TZ, QH, and CQS reviewed and revised the article. All authors read and approved the finally article.

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CONFLICT OF INTEREST

The authors declare that they have no competing interests.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

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REFERENCES

1. Pellegriti G, Frasca F, Regalbuto C, Squatrito S, Vigneri R. Worldwide increasing incidence of thyroid cancer: update on epidemiology and risk factors. *J Cancer Epidemiol.* 2013;2013:965212.
2. Ullmann TM, Gray KD, Moore MD, Zarnegar R, Fahey TJ 3rd. Current controversies and future directions in the diagnosis and management of differentiated thyroid cancers. *Gland Surg.* 2018;7(5):473-486.
3. Raue F, Frank-Raue K. Thyroid cancer: risk-stratified management and individualized therapy. *Clin Cancer Res.* 2016;22(20):5012-5021.
4. Abdullah MI, Junit SM, Ng KL, Jayapalan JJ, Karikalan B, Hashim OH. Papillary thyroid cancer: genetic alterations and molecular biomarker investigations. *Int J Med Sci.* 2019;16(3):450-460.
5. Boelaert K. The association between serum TSH concentration and thyroid cancer. *Endocr Relat Cancer.* 2009;16(4):1065-1072.
6. Hovens GC, Stokkel MP, Kievit J, et al. Associations of serum thyrotropin concentrations with recurrence and death in differentiated thyroid cancer. *J Clin Endocrinol Metab.* 2007;92(7):2610-2615.
7. Zou M, Baitei EY, Al-Rijjal RA, et al. TSH overcomes Braf(V600E)-induced senescence to promote tumor progression via downregulation of p53 expression in papillary thyroid cancer. *Oncogene.* 2016;35(15):1909-1918.
8. Davis PJ, Goglia F, Leonard JL. Nongenomic actions of thyroid hormone. *Nat Rev Endocrinol.* 2016;12(2):111-121.
9. Nisman B, Allweis TM, Carmon E, et al. Thyroid hormones, silencing mediator for retinoid and thyroid receptors and prognosis in primary breast cancer. *Anticancer Res.* 2020;40(11):6417-6428.
10. Pan JJ, Zhao L, Cheng R, Yang Y, Hu YH. Thyroid carcinoma in children and adolescents: clinical characteristics and follow-up from two centers. *J Cancer Res Ther.* 2017;13(4):715-719.
11. Blangero Y, Rabilloud M, Laurent-Puig P, et al. The area between ROC curves, a non-parametric method to evaluate a biomarker for patient treatment selection. *Biom J.* 2020;62(6):1476-1493.

12. Polat SB, Cakir B, Evranos B, et al. Preoperative predictors and prognosis of bilateral multifocal papillary thyroid carcinomas. *Surg Oncol*. 2019;28:145-149.

13. Nieto H, Boelaert K. Women in cancer thematic review: thyroid-stimulating hormone in thyroid cancer: does it matter? *Endocr Relat Cancer*. 2016;23(11):T109-T121.

14. Nisman B, Allweis TM, Carmon E, et al. Elevated free triiodothyronine is associated with increased proliferative activity in triple-negative breast cancer. *Anticancer Res*. 2021;41(2):949-954.

15. Strzałka A, Hogendorf P, Skulimowski A, Spychalski M, Strzelczyk J, Durczynski A. Thyroid hormones concentration in portal and peripheral blood in patients with pancreatic cancer: preliminary study. *Cancer Biomark*. 2020;29(3):301-306.

16. Aron M, Cote V, Tamilia M, et al. Ablative free thyroxine to thyroglobulin ratio as a predictor of differentiated thyroid cancer recurrence. *J Otolaryngol Head Neck Surg*. 2009;38(5):552-558.

17. You X, Yang S, Sui J, et al. Molecular characterization of papillary thyroid carcinoma: a potential three-lncRNA prognostic signature. *Cancer Manag Res*. 2018;10:4297-4310.

18. Pasqualetti G, Schirripa M, Dochy E, et al. Thyroid hormones ratio is a major prognostic marker in advanced metastatic colorectal cancer: results from the phase III randomised CORRECT trial. *Eur J Cancer*. 2020;133:66-73.

19. Liu C, Li L, Ha M, Qi S, Duan P, Yang K. The PI3K/Akt and ERK pathways elevate thyroid hormone receptor β1 and TRH receptor to decrease thyroid hormones after exposure to PCB153 and p,p'-DDE. *Chemosphere*. 2015;118:229–238.

20. Tam AA, Ozdemir D, Aydın C, et al. Association between preoperative thyrotrophin and clinicopathological and aggressive features of papillary thyroid cancer. *Endocrine*. 2018;59(3):565-572.

21. Fitzgerald SP, Bean NG, Falhammar H, Tuke J. Clinical parameters are more likely to be associated with thyroid hormone levels than with thyrotropin levels: a systematic review and meta-analysis. *Thyroid*. 2020;30(12):1695-1709.

22. Ke ZB, Wu YP, Huang P, et al. Identification of novel genes in testicular cancer microenvironment based on ESTIMATE algorithm-derived immune scores. *J Cell Physiol*. 2021;236(1):706-713.

23. Zhao Z, Li Y, Wu Y, Chen R. Deep learning-based model for predicting progression in patients with head and neck squamous cell carcinoma. *Cancer Biomark*. 2020;27(1):19-28.

24. Wang H, Zhou L. Random survival forest with space extensions for censored data. *Artif Intell Med*. 2017;79:52-61.

25. Pirahanchi Y, Toro F, Jialal I. *Physiology, Thyroid Stimulating Hormone*. *StatPearls*. Treasure Island (FL): StatPearls Publishing Copyright © 2022, StatPearls Publishing LLC.; 2022.

26. Song Y, Driessens N, Costa M, et al. Roles of hydrogen peroxide in thyroid physiology and disease. *J Clin Endocrinol Metab*. 2007;92(10):3764-3773.

27. Carvalho DP, Dupuy C. Thyroid hormone biosynthesis and release. *Mol Cell Endocrinol*. 2017;458:6-15.

28. Xie X, Shi X, Guan H, et al. P21-activated kinase 4 involves TSH induced papillary thyroid cancer cell proliferation. *Oncotarget*. 2017;8(15):24882-24891.

29. Lam DW, LeRoith D. Metabolic Syndrome. In: Feingold KR, Anawalt B, Boyce A, et al., eds. *Endotext*. MDText.com, Inc. Copyright © 2000–2022, MDText.com, Inc.; 2000.

30. Uzunlulu M, Telci Caklili O, Oguz A. Association between metabolic syndrome and cancer. *Ann Nutr Metab*. 2016;68(3):173-179.

31. Park JH, Choi M, Kim JH, et al. Metabolic syndrome and the risk of thyroid cancer: a nationwide population-based cohort study. *Thyroid*. 2020;30(10):1496-1504.

32. Teixeira P, Dos Santos PB, Pazos-Moura CC. The role of thyroid hormone in metabolism and metabolic syndrome. *Ther Adv Endocrinol Metab*. 2020;11:2042018820917869.

33. Ding X, Zhu CY, Li R, et al. Lower normal free thyroxine is associated with a higher risk of metabolic syndrome: a retrospective cohort on Chinese population. *BMC Endocr Disord*. 2021;21(1):39.

34. Davies T, Marians R, Latif R. The TSH receptor reveals itself. *J Clin Invest*. 2002;110(2):161-164.

35. Antunes TT, Gagnon A, Chen B, Pacini F, Smith TJ, Sorisky A. Interleukin-6 release from human abdominal adipose cells is regulated by thyroid-stimulating hormone: effect of adipocyte differentiation and anatomic depot. *Am J Physiol Endocrinol Metab*. 2006;290(6):E1140-E1144.

36. Shulman GI. Ectopic fat in insulin resistance, dyslipidemia, and cardiometabolic disease. *N Engl J Med*. 2014;371(23):2237-2238.

37. Yang X, Sun J, Han J, et al. Iodine promotes thyroid cancer development via SPANXA1 through the PI3K/AKT signalling pathway. *Oncol Lett*. 2019;18(1):637-644.

38. Gao R, Chen RZ, Xia Y, et al. Low T3 syndrome as a predictor of poor prognosis in chronic lymphocytic leukemia. *Int J Cancer*. 2018;143(3):466-477.

39. Sohn W, Chang Y, Cho YK, Kim Y, Shin H, Ryu S. Abnormal and euthyroid ranges of thyroid hormones in serum and liver cancer mortality: a cohort study. *Cancer Epidemiol Biomarkers Prev*. 2020;29(10):2002-2009.

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