Journal of Multidisciplinary Healthcare

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ORIGINAL RESEARCH

Blood Profiles in the Prediction of Radioiodine Refractory Papillary Thyroid Cancer: A Case–Control Study

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Purpose: Although most patients with papillary thyroid cancer can be cured by surgery and I-131 ablation, a small proportion will progress to radioactive iodine refractory (RAIR) thyroid cancer. The prediction of RAIR in its early stages can improve patient prognosis. The aim of this article is to evaluate the blood biomarkers in patients with RAIR and to establish a prediction model.

Patients and Methods: Data collected from patients with thyroid cancer that were enrolled from Jan. 2017 to Dec. 2021 were screened. RAIR was defined based on the criteria in the 2015 American Thyroid Association guidelines. The blood biomarkers from the study participants at three admissions timepoints (surgery and first and secondary I-131 ablations) were compared using both parametric and nonparametric tests to identify predictive factors for RAIR. Binary logistic regression analysis was used to construct a prediction model using parameters associated with surgical procedure decision. The model was then assessed with receiver operating characteristic curves.

Results: Thirty-six patients were included in the data analysis. Sixteen blood variables, including the low density lipoprotein-cholesterol-total cholesterol ratio, neutrophils, thyroglobulins, thyroglobulin antibody, thyroid peroxidase antibody, anion gap, etc., were revealed to be predictors for RAIR. The prediction model, which incorporated two parameters, reached an area under the curve of 0.861 ($p<0.001$).

Conclusion: Conventional blood biomarkers can be used in the prediction of early-stage RAIR. In addition, a prediction model incorporating multiple biomarkers can improve the predictive accuracy.

Keywords: thyroid carcinoma, radioiodine refractoriness, scoring system, multivariable, PTC

Introduction

Thyroid cancer ranks first among endocrine malignancies, with its incidence doubling since the mid-1990s.[1,2] Although most patients can be cured by surgery and postoperative thyroid-stimulating hormone (TSH) suppressing therapy,[3] approximately 20% of patients suffer from local recurrence or distant metastasis. Two-third of these individuals will develop radioactive iodine refractory (RAIR) disease, and thus have an inadequate response to I-131 remnant ablation. Generally, the 10-year-survival rate among RAIR patients is below 20%.[4] Papillary thyroid cancer (PTC) is the most common type of thyroid cancer which accounts for more than 80% of all cases.[5] In patients with RAIR, the proportion of PTC is larger than 60%.[6,7]

Currently, the diagnosis of RAIR is based on whole-body I-131 scans and the detection of local relapse and/or distant metastasis. Recurrent or metastatic lesions can be confirmed by histological or cytopathological examinations of specimens and radiological tests, such as chest computed tomography (CT), cervical magnetic resonance imaging (MRI) and whole-body bone scans.[8] More recently, positron emission tomography (PET) combined with CT has been shown to have high accuracy in the detection of metastasis.[9] These detection methods, however, require at least 1–2 rounds of radioactive iodine (RAI) treatment to

Received: 29 December 2022
Accepted: 22 February 2023
Published: 27 February 2023

make the final diagnosis of RAIR. In patients with disease progression and non-tumoricidal RAI uptake, the diagnosis is delayed even longer. All these delays subject patients to unnecessary RAI therapy and reduce their life expectancy.[10] The early-stage prediction of RAIR in patients is needed to improve the situation.

The prediction of RAIR in former studies focused on genetic mutations, especially $BRAF^{V600E}$ and TERT promoter mutations.[11,12] Recent studies have revealed some novel mutations, such as in the AXL and PLEKHS1 genes.[13,14] Other than genetic alterations, clinicopathological and ultrasound examination characteristics, including, multifocality, extrathyroidal extension (ETE), histological subtypes with aggressive behavior and the size and number of cervical lymph node metastases, were also associated with the incidence of RAIR, although the specificity was generally too low for clinical application.[7,15,16]

Laboratory examination of blood at admission is conventionally used in the preparation of patients for medical interventions. Surprisingly, blood biomarkers from conventional tests have been revealed to be associated with various cancers in clinical studies. The lipid profile, which is routinely tested in a comprehensive metabolic panel (CMP), is closely related to oral, breast, gastrointestinal and lung cancers.[17–20] Total cholesterol and low-density lipoproteins are independent predictors of a poor response to treatment and short survival, whereas high-density lipoproteins have the opposite predictive value. Similar results were also observed in patients with thyroid cancers.[21] In addition, systemic inflammatory biomarkers, such as neutrophil-lymphocyte ratio and platelet-lymphocyte ratio, have superior efficacy over single biomarkers in predicting aggressive thyroid tumor behaviors.[22,23] In addition, the coagulation status of the blood and the levels of circulating carrier protein have also been associated with thyroid cancer.[24,25] Notably, some thyroid-related blood biomarkers have been reported to have great efficacy in predicting RAIR. The most promising examples include thyroglobulin (TGB). Both TGB doubling time and the ratio of TGB at different admissions have been verified as independent predictive factors.[26,27]

The aim of this study was to evaluate the alterations in biomarkers in conventional blood tests in RAIR-PTC patients compared with non-RAIR patients. A scoring system based on the identified predictive factors was constructed and assessed.

Materials and Methods

Study Design and Patient Selection

This was a retrospective, single-center, case–control study conducted at Renmin Hospital of Wuhan University in China. The study was performed in accordance with the STROBE checklist for case–control study (version 4).[28], (Supplement 1 The Institutional Ethical Committee of Renmin hospital of Wuhan University reviewed and approved the study design (No. WDRY2021-K032) and the requirement for obtaining informed consent from the involved patients was waived due to the retrospective nature of the study design (Supplement 2. Data was processed with confidentiality. The study was conducted in accordance with the Declaration of Helsinki.[29]

The medical records of patients with PTC from Jan. 1st, 2017 to Dec. 30th, 2021 were reviewed. Patient information, including medical history, physical examinations, laboratory blood tests, histopathological results and radiological data (ultrasound examination, CT-scan examination, MRI examination, I-131 whole body scan, whole body bone scan, 18F-FDG PET/CT examination, etc.), were evaluated by two researchers (HQ. Liu & BH. Liu). Patients meeting the following criteria were included in the subsequent data extraction and analysis: a) underwent total thyroidectomy at our medical center, b) PTC confirmed by postoperative histological examination, and c) receipt of at least two radioiodine remnant ablations (Figure 1). The excluded criteria were as followed: a) <18 years of age, b) pregnant or diagnosed with other malignancies, and c) incomplete medical records. Refractoriness to RAI therapy was defined in accordance with the criteria in the 2015 American Thyroid Association (ATA) guidelines: a) the malignant/metastatic tissue does not ever concentrate RAI, b) the tumor tissue loses the ability to concentrate RAI after previous evidence of RAI-avid disease, c) RAI is concentrated in some lesions but not in others, or d) metastatic disease progresses despite significant concentration of RAI.[30] All candidates with RAI refractoriness were categorized into the RAIR group. The control group comprised 2-fold age- and sex-matched patients with RAI sensitivity who met the inclusion criteria but not the exclusion criteria.

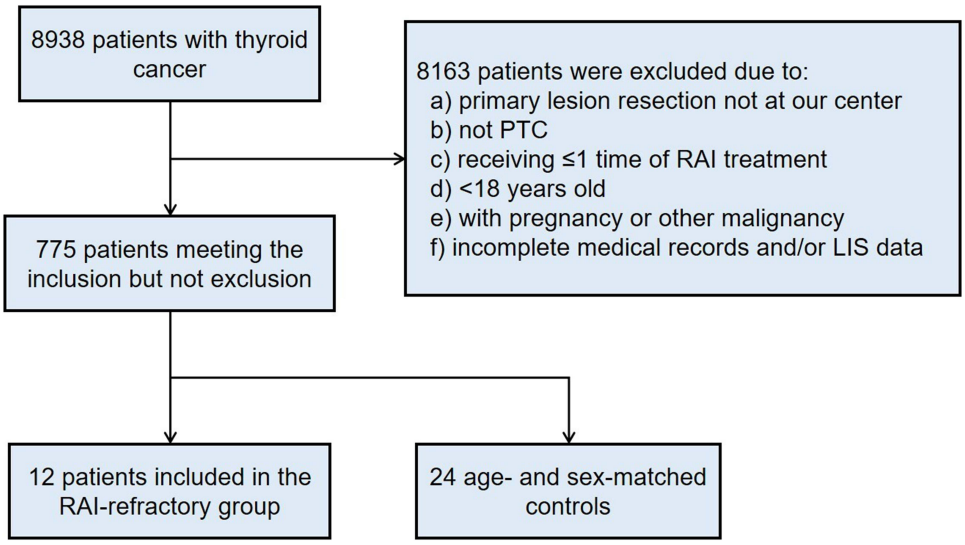

Figure 1 The flow chart of patient selection.
Abbreviations: LIS, laboratory information system; PTC, papillary thyroid cancer; RAI, radioactive iodine.

Data Collection

Data were collected from patients at admission for surgery and the first two RAI remnant ablations. The patient demographic and clinicopathologic characteristics were documented from the hospital information system and these included the following: age, sex, cumulative I-131 dose, histological type and subtype, tumor size, multifocality, ETE, cervical lymph node metastasis and distant metastasis. TNM staging was classified based on the eighth edition of the American Joint Committee on Cancer: cancer staging Manual.[31] Five to ten milliliters of blood was collected in the early morning prior to the treatment (surgery or RAI ablation) and immediately analyzed for blood biomarkers using automated analyzers (ADVIA 2400: Siemens, Germany and Sysmex XN-20 system: Kobe, Japan). The blood test results (blood cell counts, coagulation functions, thyroid function, CMP, etc.) were collected from the laboratory information system (LIS).

Statistical Analysis

Blood biomarkers with a frequency of less than 0.75 in the total patient sample were dropped from the statistical analysis. If the data fell out of detection range, data were calculated as the upper or lower limit of detection. Categorical and ordinal data were presented as numbers and percentages, while continuous data were presented as means (standard deviation, SD) or medians (interquartile range, IQR). Comparisons between the two groups were conducted using the Pearson's chi-squared test for categorical data and independent Student's *t*-test for continuous data. The equality of variances and the Gaussian distribution of the data were tested using the Levene's test and the Shapiro–Wilk test, respectively. The Mann–Whitney *U*-test was applied to evaluate the ordinal data and continuous data that did not conform to the Gaussian distribution. The blood biomarker comparison results were subjected to max-min normalization and were displayed in heatmaps using SangerBox (V3.0).[32] The average-linkage clustering with Pearson's correlation distance was applied. All tests were two-tailed and p values <0.05 were considered statistically significant.

Blood biomarkers with significant differences were processed into subsequent binary univariate logistic regression analysis with multiple imputation. Variables with a p-value <0.10 at admission for surgery were incorporated into multivariate regression analysis using conditional backward elimination procedure. The scoring system formula was constructed by multiplying the variable values with the corresponding regression coefficients and summing them. The performance of the blood biomarkers and the scoring system in the prediction of RAIR was assessed using the receiver operating characteristic (ROC) curve. The Youden index, sensitivity and specificity were then calculated at the cutoff values. The statistical analysis was performed with SPSS version 26.0 (SPSS Inc, Armonk, NY).

Results

Patient Demographic and Clinicopathological Characteristics

From Jan. 1st, 2017 to Dec. 30th, 2021, a total of 8938 individuals were diagnosed with thyroid cancer in our medical center (Figure 1). Over 91% of the total patients (8163 cases) were excluded due to a variety of reasons, including primary lesion surgery not at our center, not PTC, RAI treatment ≤1 time, incomplete medical records, etc. RAI-avidity was assessed in the remaining 775 candidates. Twelve patients were clearly categorized as RAIR based on the 2015 American Thyroid Association (ATA) criteria. The control group comprised twenty-four age- and sex-matched individuals to eliminate the confounding effects of age and sex on blood biomarker levels.

The demographic and clinicopathological characteristics were then compared between the RAIR and control groups (Table 1). The mean age was 40.58 years of age, 16.7% of participants were older than 55 years of age, and 58.3% of individuals were of female gender. Since body mass index (BMI), concomitant diseases, cigarettes and alcohol consumption are known to have an effect on circulating biomarkers,[33–36] any differences between the two groups for these conditions was assessed. No significant differences were revealed for BMI (p=0.497), hypertension (p=0.496), diabetes mellitus (p=0.453), Hashimoto's thyroiditis (p=0.708), smoking history (p=0.343) or alcohol consumption (p=0.173). The cumulative I-131 doses were much higher in the RAIR group (median: 415 mCi vs 220 mCi, p<0.001).

All included patients were diagnosed with PTC. The most common histological subtype was classic PTC, which accounted for 94.4% (34 cases) of the total sample, followed by follicular variant PTC accounting for 22.2% (8 cases) Two patients in the RAIR group were diagnosed with more aggressive subtypes (tall cell variant and solid variant).

Table 1 Demographical and Clinicopathological Characteristics of Included Patients

Parameter	Total	RAIR group	Control Group	*p*-value
Number of patients	36	12	24	
Age (years, SD)	40.58 (9.262)	40.58 (9.539)	40.58 (9.329)	1.000
≥55y (%)	6 (16.7)	2 (16.7)	4 (16.7)	1.000
Sex (female, %)	21 (58.3)	7 (58.3)	14 (58.3)	1.000
BMI (kg/m^2, SD)	24.94 (3.786)	25.56 (4.159)	24.63 (3.639)	0.497
Hashimoto's thyroiditis (%)	4 (11.1)	1 (8.3)	3 (12.5)	0.708
Hypertension (%)	5 (13.9)	1 (8.3)	4 (16.7)	0.496
Diabetes (%)	4 (11.1)	2 (16.7)	2 (8.3)	0.453
Smoking (%)	6 (16.7)	3 (25.0)	3 (12.5)	0.343
Alcohol consumption (%)	5 (13.9)	3 (25.0)	2 (8.3)	0.173
Cumulative I-131 dose (mCi) [a]	220 (220, 235)	220 (220, 220)	415 (225, 570)	**<0.001** ***
Histological subtype (%) [b]				
Classical	34 (94.4)	11 (91.7)	23 (95.8)	0.607
Follicular variant	8 (22.2)	3 (25.0)	5 (20.8)	0.777
Tall cell variant/solid variant	2 (5.6)	2 (16.7)	0 (0.0)	**0.040** *
Tumor size (cm, SD)	1.60 (0.971)	1.57 (1.053)	1.61 (0.950)	0.896
Multifocality (%)	26 (72.2)	10 (83.3)	16 (66.7)	0.293
Extrathyroidal extension (%)	19 (52.8)	7 (58.3)	12 (50.0)	0.637
TNM (%)				

(*Continued*)

Table 1 (Continued).

Parameter	Total	RAIR group	Control Group	*p*-value
T1-3	22 (61.1)	7 (58.3)	15 (62.5)	0.809
T4	14 (38.9)	5 (41.7)	9 (37.5)	
N0	3 (8.3)	1 (8.3)	2 (8.3)	1.000
N1	33 (91.7)	11 (91.7)	22 (91.7)	
M0	31 (86.1)	7 (58.3)	24 (100.0)	**0.001 ****
M1	5 (13.9)	5 (41.7)	0 (0.0)	

Notes: Bold font: significant difference. * $p<0.05$, ** $p<0.01$, *** $p<0.001$. [a]All continuous data were displayed as means and SDs except for cumulative I-131 dose, which was presented as median and IQR. [b]Seven patients had more than one subtypes in their primary lesions.
Abbreviations: BMI, body mass index; IQR, interquartile range; RAIR, radioactive iodine refractory thyroid cancer; SD, standard deviation.

However, aggressive subtypes were not found in the control group (p=0.040). Notably, seven participants were revealed to have more than one subtype of PTC in their primary lesions. No significant differences were discovered on primary tumor size (p=0.896), multifocality (p=0.293) or ETE (p=0.637). Regarding the TNM categories, there was no significant difference on the T and N categories between the two groups, whereas the RAIR group consisted of all five patients with distant metastasis (p=0.001).

Blood Biomarkers and Comparisons Between the Groups

A total of 627 blood biomarkers (209 variables at each admission) were included in the initial assessment. Three hundred and twenty items with a frequency >0.75 were reserved and processed in subsequent analyses (Supplement 3 and 4). Eight variables that conformed to Gaussian distribution showed significant differences between the RAIR and control groups (Figure 2 and Supplement 5). At the admission for total thyroidectomy, significant differences were detected in five biomarkers, including serum sodium (Na, p=0.013), low-density lipoprotein cholesterol-to-total cholesterol ratio (LDL-Ch/Tch, p=0.030), anion gap (AGPK, p=0.038), neutrophil count (Neu#, p=0.008) and white blood cell (WBC, p=0.017). Vitamin D3 (VitD3) levels at the admission for the first I-131 treatment were associated with RAIR (p=0.047).

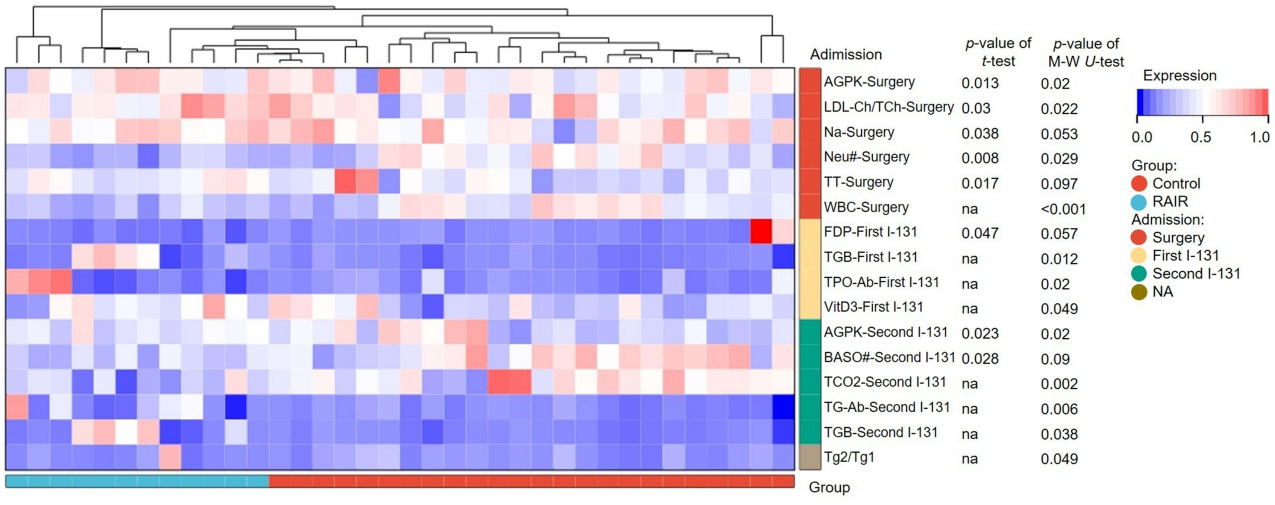

Figure 2 The heatmap of sixteen blood biomarkers with statistical significance.
Abbreviations: AGPK, anion gap; BASO#, basophil count; FDP, fibrin/fibrinogen degradation products; LDL-Ch/TCh, low-density lipoprotein cholesterol-to-total cholesterol ratio; Na, serum sodium; NA/na, not applicable; Neu#, neutrophil count; TCO2, total carbon dioxide, Tg2/Tg1, stimulated thyroglobulin at the second I-131-to-stimulated thyroglobulin at the first I-131 ratio; TG-Ab, thyroglobulin antibody; TGB, thyroglobulin; TPO-Ab, thyroid peroxidase antibody; TT, thrombin time; WBC, white blood cell; VitD3, 25-hydroxyvitamin D3.

At the second I-131 ablation, total CO_2 (TCO2, p=0.023) and basophil count (BASO#, p=0.028) were revealed to have negative correlation with RAI refractoriness.

For those that did not conform to the Gaussian distribution, the Mann–Whitney U-test revealed eight other variables with significant differences, including the following: thrombin time (TT, p<0.001) before the surgery; TGB (p=0.012), thyroid peroxidase antibody (TPO-Ab, p=0.020) and fibrin/fibrinogen degradation products (FDP, p=0.049) before the first I-131 ablation; thyroglobulin antibody (TG-Ab, p=0.002), TGB (p=0.006) and AGPK (p=0.038) before the second I-131 ablation; and the ratio of stimulated thyroglobulin at the second I-131-to-The stimulated thyroglobulin at the first I-131 (Tg2/Tg1, p=0.049). Of note, FDP, BASO#, TCO2, Neu# and WBC were inversely associated with RAIR, whereas the other biomarkers showed positive correlations.

Construction of the Scoring System

The odds ratios (OR) and their 95% confidence intervals (95% CI) for the sixteen variables were then calculated using univariate logistic regression analysis (Table 2). Significant differences were revealed in four of the six biomarkers at admission for surgery, including AGPK (p=0.049), LDL-Ch/TCh (p=0.023), Na (p=0.024) and TT (p=0.024). Two variables at surgery were on the borderline of statistical significance (Neu#: p=0.054, WBC: p=0.072). Among the other parameters, only TCO2 at the second I-131 therapy was inversely associated with RAIR (p=0.033). Since these parameters at other admission timepoints could not be collected in the very early stages, all six biomarkers at surgery were input into the multivariate logistic regression model.

Table 2 Logistic Regression Analysis for the Association Between Blood Biomarkers and RAIR

Variable	Admission	Univariate		Multivariate		
		OR (95% CI)	***p*-value**	**β value**	**OR (95% CI)**	***p*-value**
AGPK	Surgery	1.392 (1.001, 1.936)	**0.049** *			0.161
LDL-Ch/TCh	Surgery	4.606e+6 (8.277, 2.564e+12)	**0.023** *	19.750	3.778e+8 (31.953, 4.466e+15)	**0.017** *
Na	Surgery	1.549 (1.060, 2.262)	**0.024** *			0.246
Neu#	Surgery	0.480 (0.228, 1.012)	0.054			0.535
TT	Surgery	2.125 (1.106, 4.084)	**0.024** *			0.274
WBC	Surgery	0.622 (0.370, 1.044)	0.072	−0.627	0.534 (0.292, 0.979)	**0.042** *
FDP	First I-131	0.924 (0.692, 1.233)	0.591			
TGB	First I-131	1.042 (0.982, 1.106)	0.172			
TPO-Ab	First I-131	1.003 (1.000, 1.006)	0.083			
VitD3	First I-131	1.130 (0.994, 1.284)	0.061			
AGPK	Second I-131	1.356 (0.982, 1.874)	0.065			
BASO#	Second I-131	0.000 (0.000, 105.915)	0.077			
TCO2	Second I-131	0.684 (0.482, 0.969)	**0.033** *,a			
TG-Ab	Second I-131	1.043 (0.992, 1.097)	0.101			
TGB	Second I-131	1.034 (0.962, 1.111)	0.367			
Tg2/Tg1	NA	3.861 (0.683, 21.808)	0.126			

Notes: Bold font: significant difference. * p<0.05. [a]The data of TCO2 at the admission for the second I-131 ablation were not input into multivariate analysis since it could not be acquired in the early stage of treatment.

Abbreviations: AGPK, anion gap; BASO#, basophil count; CI, confidence interval; FDP, fibrin/fibrinogen degradation products; LDL-Ch/TCh, low density lipoprotein cholesterol-to-total cholesterol ratio; Na, serum sodium; NA, not applicable; Neu#, neutrophil count; OR, odd ratio; RAIR, radioactive iodine refractory thyroid cancer; TCO2, total carbon dioxide, Tg2/Tg1, stimulated thyroglobulin at the second I-131-to-stimulated thyroglobulin at the first I-131 ratio; TG-Ab, thyroglobulin antibody; TGB, thyroglobulin; TPO-Ab, thyroid peroxidase antibody; TT, thrombin time; WBC, leukocyte; VitD3, 25-hydroxyvitamin D3.

The subsequent multivariate logistic regression analysis showed that the LDL-Ch/TCh ratio correlated positively with the incidence of RAIR (β=19.750, p=0.017), while the WBC levels at surgery were inversely associated with the condition (β=−0.627, p=0.042). The two regression coefficients were rounded to one significant digit and the formula for the prediction of RAIR was constructed as following: Score = 20 × LDL-Ch/TCh - 0.6 × WBC.

Performance of the Prediction Model

The scores of the RAIR group were higher than those of the control group (median: 8.753 vs 6.723, p<0.001) (Figure 3). ROC curve was applied to evaluate the performance of the six included variables at surgery and the scoring system (Figure 4 and Table 3). The prediction model incorporating the LDL-Ch/TCh and WBC had the largest area under the

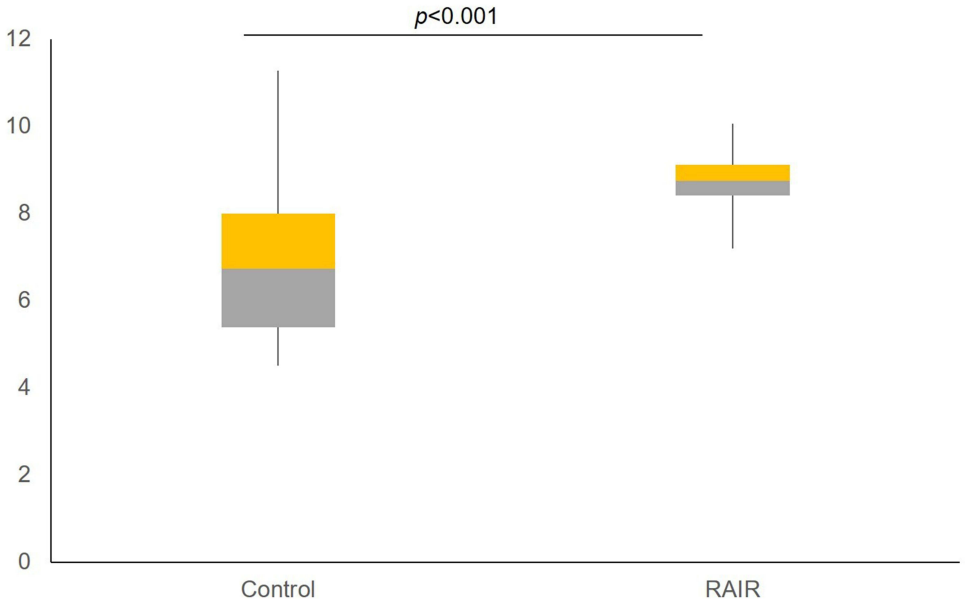

Figure 3 The comparison of scores between the two groups. The medians and interquartile ranges were displayed here. The Mann–Whitney *U*-test was applied.
Abbreviation: RAIR, radioactive iodine refractoriness.

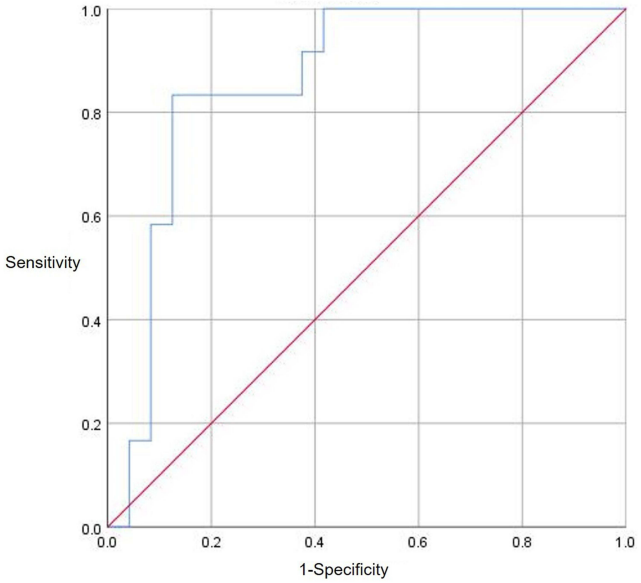

Figure 4 The ROC curve of the scoring system.

Table 3 Predictive Accuracy of the Blood Markers and the Scoring System Based on the ROC Curve

Variable	AUC	*p*-value	Cut-off Value	Sensitivity	Specificity	Youden Index
AGPK	0.701	0.052	12.17	0.833	0.500	0.333
LDL-Ch/TCh	0.759	**0.012 ***	0.551	0.917	0.625	0.542
Na	0.740	**0.021 ***	142.25	0.583	0.875	0.458
Neu# [a]	0.726	**0.029 ***	4.205	0.583	1.000	0.583
TT	0.858	**0.001 ****	17.15	1.000	0.750	0.750
WBC [a]	0.674	0.093	7.355	0.458	1.000	0.458
Scoring system	0.861	**<0.001 *****	8.341	0.833	0.875	0.708

Notes: Bold font: significant difference. *p<0.05, **p<0.01, ***p<0.001. [a]Neutrophil and leukocyte count were negatively correlated with the RAIR. Hence their AUCs were calculated on the condition that the control group was defined as the state variable.
Abbreviations: AGPK, anion gap; AUC, area under the curve; LDL-Ch/TCh, low density lipoprotein cholesterol-to-total cholesterol ratio; Na, serum sodium; Neu#, neutrophil count; ROC, receiver operating characteristics; TT, thrombin time; WBC, leukocyte.

curve (AUC) in comparison with models having single predictors (AUC=0.861, p<0.001). A cutoff value of 8.34 was found to have the best prediction accuracy. The sensitivity, specificity and Youden index at this point were 0.833, 0.875 and 0.708, respectively.

Discussion

In most cases, patients with ATA intermediate- or high-risk level PTCs undergo at least two RAI remnant ablations after total or near total thyroidectomy. Complete blood counts (CBCs), CMPs, thyroid function tests and coagulation function tests are routinely performed to evaluate basic patient conditions before medical interventions. The current study revealed that sixteen blood biomarkers determined at three different admission visits can serve as predictors in patients with RAIR-PTC. Six of these biomarkers can be detected before the thyroid operations. Among them, AGPK, LDL-Ch/TCh and serum Na can be evaluated with CMPs. WBC and neutrophil counts are conventional parameters in CBCs. TT, the last of the six biomarkers, can be collected from coagulation function tests. Of great interest, none of the biomarkers in the thyroid function test at admission for surgery was revealed to be associated with iodine refractoriness. In the subsequent RAI remnant ablations, parameters in CMP (VitD3, AGPK and TCO2), CBC (basophil), coagulation function test (FDP) and thyroid function test (TPO-Ab, TG-Ab, TGB and Tg2/Tg1) were revealed to have significantly different values in patients with RAIR.

Lipid metabolism has been confirmed to have an effect on tumorigenesis, progression and invasion.[37,38] The underlying mechanisms may be Akt (protein kinase B) phosphorylation and oxidative stress induced by elevated LDL.[37,39] It is well-known that LDL is the major plasma carrier of cholesterol from the liver to peripheral tissues. In a previous study by Sahin et al, higher serum LDL levels were observed in patients with PTC than those seen in normal volunteers.[40] Given that LDL-receptor-related protein 4 (LRP4) is upregulated in PTC cells,[41,42] we hypothesize that the elevated cholesterol influx in tumor cells promotes dedifferentiation. In this study, we also demonstrated that LDL-Ch/TCh was a great biomarker in predicting RAIR-PTC.

AGPK is an electrolytic biomarker that is calculated by subtracting the sum of chloride and bicarbonate from serum sodium and potassium. In a national database study, Verma and et al have discovered that the mortality in cancer patients increased by 8% with each unit increase in AGPK.[43] Although the phenomenon is difficult to explain, we further noticed the elevated Na and reduced TCO2 levels in RAIR patients. Electrolytic dysfunction is common in cancer patients with paraneoplastic syndromes. The most frequent type of electrolytic dysfunction is, however, hyponatremia. Elevated sodium is more likely to be interpreted as an indicator of poor prognosis and high mortality, as suggested by studies on other cancer types.[44,45]

The reduction in WBCs in the RAIR patients may be attributed to the decrease in blood neutrophils. Aside from its function in innate immunity, neutrophils serve as important mediators in carcinogenesis and metastasis. A high

neutrophil-lymphocyte ratio and tumor-associated neutrophils in the stroma of cancer tissues indicate high aggressiveness and a short-life expectancy.[46] Interestingly, the effects of neutrophils on cancer cells are dual. They can also exert an inhibitory effect on tumor growth by antibody-dependent cellular cytotoxicity.[47] Primary tumor cells secrete C-motif chemokine ligand 2 (CCL2) and granulocyte colony-stimulating factor (G-CSF), which subsequently activate the neutrophils. These cells exert antimetastatic functions by producing H_2O_2 and recruiting cytotoxic T-cells.[48] In addition, considering that patients with RAIR were more likely to have distant metastasis, the reduction of WBC might be due to cancer-associated consumptive disease. The elongation of thrombin time, which might result from reduced production of fibrinogen, also supports this hypothesis.

Thyroid-related circulating biomarkers are closely associated with RAIR. TGB is a thyroid-specific tumor-associated antigen. Either normal or malignant thyroid cells can produce TGB and release it into the circulation. In patients with a thyroid cancer history, TGB is considered a rough estimate of tumor burden. An elevation of TGB strongly indicates local recurrence or distant metastasis. Since the baseline TBG levels range widely in PTC patients, the TBG doubling time is a more specific predictor in monitoring PTC. Wassermann et al conducted a retrospective study in 153 patients with metastatic differentiated thyroid cancer.[26] A TBG doubling time <1 year was a hazardous factor indicating decreased survival. The predictive value of TGB doubling time was confirmed by other clinical studies.[49,50] Another method to normalize the individual TGB levels is to calculate the Tg2/Tg1. Similar to TGB doubling time, an elevation of Tg2/Tg1 is a strong indicator of PTC progression, as revealed in a retrospective single-center study by Wang et al.[27] Except for TGB, TG-Ab and TPO-Ab are also correlated with RAIR. TG-Ab and TPO-Ab are closely related to Hashimoto's thyroiditis.[51] TG-Ab has been revealed as a confounding factor for the evaluation of TGB since it can competitively bind with TGB in any immunological assay.[52] Recent studies found that postoperative TG-Ab levels were positively correlated with the remnant after I-131 ablation.[53,54] In contrast, TPO-Ab has been less studied in the field of I-131 therapy. In most cases, TPO-Ab was tested to evaluate the incidence of Hashimoto's thyroiditis after I-131 ablation.[55,56] In this study, the slight elevation of TPO-Ab might be attributed to the increasing apoptosis of thyroid cancer cells and the subsequent release of TPO into circulation due to I-131 damage.

Previous studies on the prediction of RAIR focused on gene mutations. The unsatisfactory effect of I-131 treatment may be partially explained by reduced expression of sodium-iodide symporter (NIS) or its dislocation to nonfunctional intracellular space.[4] BRAF and TERT promoter mutations influence translation and location via the mitogen-activated protein kinase (MAPK) pathway.[16,57] The genetic duet of $BRAF^{V600E}$ and TERT promoter mutations in primary lesions of PTCs has been reported to robustly predict the RAIR.[11] A positive predictive value of 97.4% and a negative predictive value of 52.8% were reached in this study. Although other studies in this field also confirmed the relationship between these two mutations and RAIR, the predictive accuracy varied in different studies.[6,12,14,58] The discrepancy might be explained by different patient selection and sequencing techniques. Since targeted sequencing is costly in most medical institutions, other researchers tried to use clinicopathological and ultrasound examination characteristics to predict the RAIR. Aggressive histopathological subtypes (tall cell variant PTC, diffuse sclerosing variant PTC, hobnail variant PTC, Hurthle cell carcinoma, poorly differentiated thyroid cancer, etc.), ETE, multifocality and large cervical lymph node metastasis were revealed to be predictive variables for RAIR.[7,11,59,60] However, the sensitivity and specificity were generally low and were not satisfactory in clinical practice.

There are some limitations in this study. First, due to the intrinsic weakness of the retrospective study design, avoidance of selection bias is challenging. The selection protocol in this study entailed at least two I-131 whole-body scans to avoid false negative RAI uptake. Some patients with poor response to I-131 may turn to other treatments after the first RAI therapy. Since the study was conducted in a single medical center, the generalization of our hypothesis is limited. In addition, the relatively small sample size inevitably undermined the reliability of the conclusions. In addition, the prediction model in this study was not tested with a validation set due to the limited number of included patients.

Conclusion

In summary, this study is, to our knowledge, the first investigation of the association between conventional blood biomarkers and RAIR-PTC. Sixteen variables were revealed to have predictive value in the diagnosis of RAIR and six of

them could be collected before the thyroidectomy. Multicenter and prospective studies with larger sample sizes are required to verify our conclusions.

Abbreviations

95% CI, 95% confidence interval; AGPK, anion gap; Akt, protein kinase B; ATA, American Thyroid Association; AUC, area under the curve; BASO#, basophil count; BMI, body mass index; CBC, complete blood count; CCL2, C-motif chemokine ligand 2; CMP, comprehensive metabolic panel; CT, computed tomography; ETE, extrathyroidal extension; FDP, fibrin/fibrinogen degradation products; G-CSF, granulocyte colony stimulating factor; IQR, interquartile range; LDL-Ch/TCh, low density lipoprotein cholesterol-to-total cholesterol ratio; LIS, laboratory information system; LRP4, LDL receptor-related protein 4; MAPK, mitogen-activated protein kinase; MRI, magnetic resonance imaging; Na, serum sodium; Neu#, neutrophil count; NIS, sodium-iodide symporter; OR, odds ratio; PET, positron emission tomography; PTC, papillary thyroid cancer; RAI, radioactive iodine; RAIR, radioactive iodine refractoriness; ROC, receiver operating characteristic; SD, standard deviation; TCO2, total carbon dioxide, Tg2/Tg1, stimulated thyroglobulin at the second I-131-to-stimulated thyroglobulin at the first I-131 ratio; TG-Ab, thyroglobulin antibody; TGB, thyroglobulin; TPO-Ab, thyroid peroxidase antibody; TT, thrombin time; WBC, white blood cell; VitD3, 25-hydroxyvitamin D3.

Data Sharing Statement

All data generated or analyzed during this study are available from the corresponding author on reasonable requests.

Acknowledgments

We wish to thank Professor Jun Liang for her assistance and advice on the patient selection protocol.

Author Contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

Funding

This research was supported by the grants from Beijing Xisike Clinical Oncology Research Foundation (Y-SY201901-0189), the Fundamental Research Funds for the Central Universities (2042019kf0229), the Science and Technology Major Project of Hubei Province (Next-Generation AI Technologies) (2019AEA170).

Disclosure

Prof. Dr Chuang Chen reports grants from Beijing Xisike Clinical Oncology Research Foundation, grants from Fundamental Research Funds for the Central Universities, grants from Science and Technology Major Project of Hubei Province, during the conduct of the study. The authors report no conflicts of interest in this work.

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