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Exploration of risk factors for the occurrence and recurrence of papillary thyroid carcinoma with Hashimoto's thyroiditis based on next-generation sequencing

Wanxue Zhang¹ and Bin Liu^{1*}

Abstract

Objectives To investigate the risk factors for the occurrence and recurrence of Hashimoto's thyroiditis (HT) combined with papillary thyroid carcinoma (PTC) using Next-Generation Sequencing (NGS).

Methods A total of 249 patients who underwent thyroid cancer surgery at the First Affiliated Hospital of Anhui Medical University from October 2020 to December 2020 were included in this study. They were divided into two groups: HT and non-HT (NHT) groups based on whether they were diagnosed with HT or not. Clinical data, NGS results, and 4-year follow-up recurrence were collected and analyzed using binary logistic regression and COX regression analysis to identify factors influencing the occurrence and recurrence of PTC with HT.

Results Patients in the HT group had a higher percentage of low age, multifocality, high TgAb, and RET rearrangement compared to the NHT group. However, they had a lower rate of extrathyroidal extension (ETE), lymph node metastasis (LNM), and BRAF mutation ($P < 0.05$). Among patients with RET rearrangement in the HT group, there was a higher rate of LNM and recurrence ($P < 0.05$). Patients with BRAF mutation in the HT group were more likely to be male and have multifocal tumors ($P < 0.05$). Binary logistic regression analysis showed that multifocality, RET rearrangement, low age, and lymph node negativity were risk factors for HT combined with PTC. Univariate COX analysis revealed that multifocality, LNM, ETE, recurrence risk stratification, TSH, RET rearrangement, and the interaction between RET rearrangement and Hashimoto's effect were risk factors for recurrence after PTC. Multifactorial COX regression analysis showed that ETE and recurrence risk stratification were risk factors for recurrence after PTC surgery.

Conclusions Multifocality, high TgAb, low age, and lymph node negativity can assist in the preoperative diagnosis of HT combined with PTC. BRAF mutations are less common in HT combined with PTC but do not significantly affect recurrence. Patients with RET rearrangement in addition to HT have a higher risk of recurrence, and special attention should be paid to intraoperative clearance in these patients.

Keywords Papillary thyroid carcinoma, Hashimoto's Disease, Next Generation Sequencing, RET rearrangement

Introduction

Hashimoto's thyroiditis (HT) is an autoimmune inflammatory disease of the thyroid gland and the leading cause of hypothyroidism. Papillary Thyroid Carcinoma (PTC) is the most prevalent subtype of thyroid cancer and has a favorable prognosis. However, it can also present early with cervical LNM, and distant metastasis can occur in

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later stages of disease progression. Since Dailey et al. [1] first found an association between HT and PTC in 1955, more in-depth studies have been carried out. These studies suggest that HT and PTC may have a better clinical prognosis [2]. However, there are still some articles [3] pointing out that HT combined with PTC has a higher malignancy and LNM rate. Other studies [4] have found that PTC combined with HT may have a centralized regional neck lymph node metastasis with a lower probability, but it is a risk factor for cervical lateral lymph node metastasis (LLNM). The pathogenesis of HT combined with PTC is still in the exploratory stage, and it is unclear whether the relationship between the two is causal or incidental [5–7]. Additionally, the reactive hyperplasia of lymph nodes induced by HT [8] increases the difficulty of preoperative assessment of LNM and intraoperative lymph node clearance. This may lead to residual cancer and a higher risk of recurrence. Postoperative complications, such as postoperative hypocalcemia and hoarseness [9], can also increase the probability of recurrent secondary surgery due to scar proliferation and tissue adhesions from previous neck surgery. The guidelines have not yet provided clear guidance on the extent of surgery for patients with PTC combined with HT. This is especially true for whether unilateral total excision is necessary and whether lymph node dissection should be performed for those with suspicious cervical lymph node enlargement suggested by preoperative imaging data. Furthermore, many studies have shown that HT is often combined with microscopic and occult cancers [10–13], which can lead to missed diagnosis and missed resection. Therefore, postoperative pathologic diagnosis should be assisted by a combination of comprehensive genetic testing. The presence of a dense lymphocytic infiltration in HT may reduce the sensitivity of methods for detecting relevant mutations in thyroid cytology specimens [14, 15]. Next-Generation Sequencing (NGS), a powerful tool for detecting mutations in thyroid cytology specimens, is a next-generation sequencing technology with high throughput, high depth, and high sensitivity. It has been widely used in basic or clinical studies of various cancers [16]. The aim of this study was to use NGS technology to detect relevant gene mutations, explore the risk factors for the occurrence and recurrence of HT combined with PTC, and provide more references for the development of clinical surgical protocols.

Materials and methods

General information

This study is a retrospective cohort study that consecutively included 249 patients who underwent thyroid cancer surgery from October 2020 to December 2020 in the First Affiliated Hospital of Anhui Medical University.

Clinical data including age, gender, thyroid function, tumor diameter, postoperative pathology with or without ETE number of cancer foci, and presence of LNM were collected from the patients.

Inclusion criteria: ① all met the clinical diagnostic criteria of PTC and HT and were confirmed by postoperative pathological examination; ② all received surgery and other related treatments for the first time; ③ all did not have the combination of cardiovascular, cerebral vascular, hepatic, renal, and other important organs and tissues diseases; ④ all voluntarily participated in the study and signed the informed consent.

Exclusion criteria: ① Combined with other thyroid diseases such as primary hyperthyroidism, positive thyroid stimulating hormone receptor antibody or diffuse enlargement of the thyroid gland; ② Previous thyroid surgery or combined with hyperthyroidism; ③ Combined with follicular or medullary carcinoma and other types of thyroid cancer; ④ Combined with immune, hematological diseases or other malignant tumors; ⑤ Clinical information is incomplete or the degree of cooperation of the patients is poor.

Diagnostic criteria: Diagnostic criteria of HT: thyroid tissue is diffusely enlarged and hard in consistency; preoperative ultrasound shows that the thyroid gland is diffusely echogenic; preoperative thyroid function shows that thyroid peroxidase antibody (TPOAb) and thyroglobulin antibody (TgAb) are elevated; and pathological findings suggest HT. Diagnostic criteria for thyroid cancer: preoperative puncture pathology or postoperative pathology suggestive of thyroid cancer. TNM staging was based on the staging definition criteria in the eighth edition of the American Joint Committee on Cancer (AJCC).

All patients signed an informed consent form, and the study was approved by the Ethics Committee of the First Affiliated Hospital of Anhui Medical University.

Tumor tissue gene sequencing

Fresh tissue specimens obtained from surgery were immediately frozen in liquid nitrogen and stored at -70°C until sequencing. DNA was extracted using HiPure FFPE DNA kit and RNA was extracted using RNAPure FFPE kit, and the extracted DNA and RNA samples were used for library construction, and NGS technology was applied for nucleic acid sequencing, and the data were filtered and compared with the nucleic acid sequences in the libraries. We analyzed and detected all the hotspot exon regions and some introns of human BRAF, RET, EGFR, FGFR, HRAS, KRAS, NRAS, NTRK, TERT and other genes.

Postoperative follow-up

After surgery, all patients took levothyroxine tablets for long-term TSH suppression therapy, and none of them underwent chemotherapy or radiation therapy after surgery. The patients were followed up at regular intervals, every 1 month for the first 3 months, every 3 months for the first year, and every 6 months thereafter. Follow-up visits were mainly in outpatient clinics and by telephone, and included thyroid function and thyroid ultrasound. Patients who did not have regular follow-up and did not take medication regularly were regarded as lost patients, and if the follow-up patient was diagnosed with recurrent/persistent thyroid cancer, the follow-up would end.

Diagnostic criteria for recurrent/persistent thyroid cancer [17]: reappearance of biochemical or imaging abnormalities after initial treatment after negative imaging performance and failure to measure thyroglobulin (Tg) on stimulation with TSH, and the site of recurrence can be a remnant gland, or extra-glandular in the form of lymph node metastasis or distant metastasis.

Statistical analysis

This study used SPSS version 25.0 to statistically analyze the test results and clinicopathological characteristics. Categorical variables were analyzed using the chi-square test or Fisher's exact test, and expressed as the number of cases and percentage (%); continuous variables were analyzed using the t-test or Mann-Whitney U-test, and normal independent samples were described by mean \pm standard deviation, and non-normal measures were described by quartiles M (Q1-Q3); the factors affecting the occurrence of HT combined with PTC were analyzed using binary logistic regression; Cox regression was used to analyze the factors affecting recurrence; $P < 0.05$ was statistically different from each other. analysis; Cox regression method was used to analyze the factors affecting recurrence; $P < 0.05$ was statistically different.

Results

Comparison of clinical features, thyroid function indexes and NGS results between the HT and NHT groups

In the NGS results, gene mutations were detected in 195 patients (78.31%) out of 249 PTC specimens. Among these 249 patients, 159 patients (63.86%) had single gene mutations and 36 patients (14.46%) had 2 or more gene mutations. Among the detected genes, BRAF gene had the highest mutation rate (164 cases) with a mutation rate of 65.86%, and the rest were, in order, RET (20 cases), TERT (13 cases), NRAS/HRAS, TP53 mutation (5 cases), TSHR (3 cases), and PIK3 CA (3 cases) (the remaining

mutated genes were less than or equal to 1 case were not discussed).

Compared with the NHT group, patients in the HT group were younger, had higher TgAb, less ETE, more multifocality, lower LNM rate, lower BRAF mutation rate, and higher RET rearrangement rate ($P < 0.05$). There were no statistical differences between the two groups in gender composition, maximum tumor diameter, recurrence rate, TERT mutation, NRAS/HRAS mutation, TSHR mutation, TP53 mutation, PIK3 CA mutation, and NTRK1 mutation ($P > 0.05$). (Fig. 1, Table 1).

Comparison of clinical characteristics of patients with RET rearrangements and BRAF mutations in the HT group

2.2.1 Compare the clinical characteristics of patients with positive and negative RET rearrangement in the HT group. Patients with RET rearrangement had a higher incidence of LNM and LLNM and a higher recurrence rate ($P < 0.05$), while all other clinical characteristics were not statistically significantly different from those of the NRET group (Table 2).

2.2.2 Compare the clinical characteristics of BRAF mutation positive and negative patients in the HT group. Patients who developed BRAF mutations were more male and multifocal ($P < 0.05$), and other clinical features and the presence or absence of LNM and recurrence rate were not statistically significantly different from those of the NBRAF group (Table 3).

Binary logistic regression analysis of independent risk factors causing HT combined with PTC

According to the assignment table (Table 4), whether combined HT was the dependent variable, age, whether multifocal, whether LNM, whether ETE, TgAb, BRAF mutation, and RET rearrangement were used as the independent variables, and the analysis of regression model showed that multifocal and RET rearrangement were the significant positive influences of HT combined with PTC, and advanced age and LNM had a significant negative influence on them, and ETE, TgAb, and BRAF mutation did not constitute a statistically significant effect on it. (Fig. 2).

Analysis of recurrence

All 249 patients were effectively followed up, and there were no lost patients, with a follow-up rate of 100.00%. The follow-up was for four years with a cut-off date of October 2024, and the median follow-up time was 38.1 months. Overall recurrent/persistent thyroid cancer occurred in 21 cases with a recurrence/persistence rate of 8.43%. The initial surgical approach and site of recurrence are shown in Table 5.

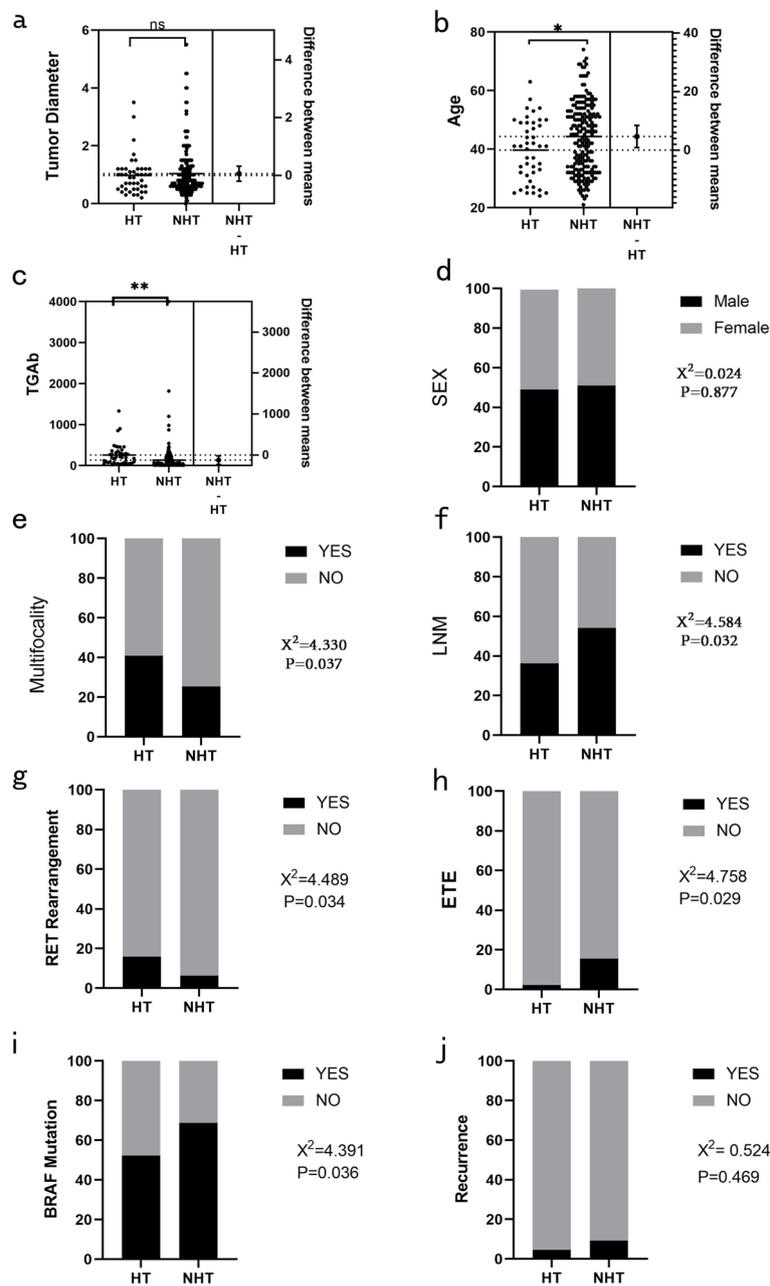


Fig. 1 (a-j) Comparison of basic information between the Hashimoto group and the control group

Log-rank test showed that the overall recurrence rate was 4.55% (2/44) in the HT group and 9.27% (19/205) in the NHT group, and the difference was not statistically significant ($\chi^2 = 0.772, P = 0.380$); the BRAF mutation-positive recurrence rate was 4.35% (1/23) in the HT group, and the overall recurrence rate was 4.76% (1/21) in the NHT group. The difference was not statistically significant ($\chi^2 = 0.007, P = 0.935$); whereas the positive recurrence rate of RET rearrangement in the HT group

was 28.57% (2/7), and the total recurrence rate in the NHT group was 0% (0/37), and the difference was statistically significant ($\chi^2 = 11.403, P = 0.001$);

Unifactorial and multifactorial COX regression analysis of independent risk factors causing HT combined with PTC

2.5.1 Unifactorial cox regression analysis of the influencing factors of recurrence in patients in the HT group suggested that tumor diameter and ETE ($P < 0.05$) were the

Table 1 Comparison of basic information between HT group and NHT group

| Characteristics | HT (n = 44) | NHT (n = 205) | t/Z (X ²) | P-value |
|---------------------|-----------------|--------------------|-----------------------|---------|
| Age | 41 (31.25,49) | 45 (33.5,53) | 2.431 | 0.016 |
| Sex | | | (0.024) | 0.877 |
| Male | 13 (29.55%) | 63 (30.73%) | | |
| Female | 31 (70.45%) | 142 (69.27%) | | |
| ETE | | | (4.758) | 0.029 |
| Yes | 1 (2.27%) | 32 (15.61%) | | |
| No | 43 (97.73%) | 173 (84.39%) | | |
| Tumor Diameter (cm) | 0.95 (0.5,1.2) | 0.8 (0.5,1.2) | 0.457 | 0.648 |
| Multifocality | | | (4.330) | 0.037 |
| No | 26 (59.09%) | 153 (74.63%) | | |
| Yes | 18 (40.91%) | 52 (25.37%) | | |
| LNM | | | (4.584) | 0.032 |
| No | 28 (63.64%) | 94 (45.85%) | | |
| Yes | 16 (36.36%) | 111 (54.15%) | | |
| TGAb | 269 (61.95,417) | 14.73 (4.98,28.07) | - | <0.001 |
| BRAF Mutation | | | (4.391) | 0.036 |
| Yes | 23 (52.27%) | 141 (68.78%) | | |
| No | 21 (47.73%) | 64 (31.22%) | | |
| RET rearrangement | | | (4.489) | 0.034 |
| Yes | 7 (15.91%) | 13 (6.34%) | | |
| No | 37 (84.09%) | 192 (93.66%) | | |
| TERT Mutation | | | (<0.001) | 1.000 |
| Yes | 2 (4.55%) | 11 (5.37%) | | |
| No | 42 (95.45%) | 194 (94.63%) | | |
| NRAS/HRAS Mutation | | | | 0.594 |
| Yes | 0 (0.00%) | 6 (2.93%) | | |
| No | 44 (100.00%) | 199 (97.07%) | | |
| TSHR Mutation | | | (<0.001) | 1.000 |
| Yes | 1 (2.27%) | 2 (0.98%) | | |
| No | 43 (97.73%) | 203 (99.02%) | | |
| TP53 Mutation | | | | 0.590 |
| Yes | 0 (0.00%) | 5 (2.44%) | | |
| No | 44 (100.00%) | 200 (97.56%) | | |
| PIK3CA Mutation | | | (2.812) | 0.140 |
| Yes | 2 (4.55%) | 1 (0.49%) | | |
| No | 42 (95.45%) | 204 (99.51%) | | |
| Recurrence | | | 0.524 | 0.469 |
| Yes | 2 (4.55%) | 19 (9.27%) | | |
| No | 42 (95.45%) | 186 (90.73%) | | |

ETE Extrathyroidal extension, LNM Lymph node metastasis

correlates of postoperative recurrence in the HT group, whereas gender, TSH, age, the number of cancerous foci, LNM, BRAF mutation, and RET rearrangement had nothing to do with them. (Table 6).

2.5.2 Univariate cox regression analysis of the influencing factors of overall patient recurrence suggested that multifocal, LNM, ETE, higher recurrence risk stage, higher TSH, positive RET rearrangement, and RET

rearrangement interacting with Hashimoto's effect were the correlates of postoperative recurrence after PTC ($P < 0.05$), whereas gender, age, tumor diameter, simple combined Hashimoto's, positive BRAF mutation, BRAF-interacting RET and BRAF-interacting bridging effects were not related to them ($P > 0.05$). (Table 7).

2.5.3 Multifactorial COX analysis, 6 variables of TSH, multifocal, LNM, ETE, recurrence risk stage, and RET

Table 2 Comparison of basic information between RET rearrangement-positive and negative groups in the HT group

| Characteristics | RET Rearrangement | | t/Z (χ^2) | P-value |
|---------------------|-------------------|-------------------|------------------|---------|
| | Positive (n = 7) | Negative (n = 37) | | |
| Age | 34 (25,50) | 41 (32,49) | -0.627 | 0.531 |
| Sex | | | (<0.001) | 1.000 |
| Male | 2 (28.57%) | 11 (29.73%) | | |
| Female | 5 (71.43%) | 26 (70.27%) | | |
| ETE | | | | 0.159 |
| Yes | 1 (14.29%) | 0 (0.00%) | | |
| No | 6 (85.71%) | 37 (100.00%) | | |
| Tumor Diameter (cm) | 1 (0.6,3) | 0.9 (0.5,1.2) | -0.903 | 0.377 |
| Multifocality | | | (<0.001) | 1.000 |
| No | 4 (57.14%) | 22 (59.46%) | | |
| Yes | 3 (42.86%) | 15 (40.54%) | | |
| LNM | | | | <0.001 |
| No | 0 (0.00%) | 28 (75.68%) | | |
| Yes | 7 (100.00%) | 9 (24.32%) | | |
| LLNM | | | (5.750) | 0.016 |
| No | 5 (71.43%) | 30 (81.08%) | | |
| Yes | 2 (28.57%) | 7 (18.92%) | | |
| Recurrence | | | | 0.022 |
| Yes | 2 (28.57%) | 0 (0.00%) | | |
| No | 5 (71.43%) | 37 (100.00%) | | |

ETE Extrathyroidal extension, LNM Lymph node metastasis, LLNM Lateral neck lymph node metastasis

Table 3 Comparison of basic information between BRAF mutation positive and negative groups in HT group

| Characteristics | BRAF Mutation | | t/Z (χ^2) | P-value |
|---------------------|-------------------|-------------------|------------------|---------|
| | Positive (n = 23) | Negative (n = 21) | | |
| Age | 37 (27,47) | 42 (34,50) | -1.558 | 0.112 |
| Sex | | | (6.006) | 0.014 |
| Male | 11 (47.83%) | 2 (9.52%) | | |
| Female | 12 (52.17%) | 19 (90.48%) | | |
| ETE | | | | 1.000 |
| Yes | 1 (4.35%) | 0 (0.00%) | | |
| No | 22 (95.65%) | 21 (100.00%) | | |
| Tumor Diameter (cm) | 1 (0.4,1.2) | 0.9 (0.55,1.2) | -0.756 | 0.450 |
| Multifocality | | | (4.859) | 0.027 |
| No | 10 (43.47%) | 16 (76.19%) | | |
| Yes | 13 (56.52%) | 5 (23.80%) | | |
| LNM | | | (2.736) | 0.098 |
| No | 12 (52.17%) | 16 (76.19%) | | |
| Yes | 11 (47.83%) | 5 (23.80%) | | |
| LLNM | | | (0.243) | 0.622 |
| No | 16 (69.57%) | 16 (76.19%) | | |
| Yes | 7 (30.43%) | 5 (23.80%) | | |
| Recurrence | | | (<0.001) | 1.000 |
| Yes | 1 (4.35%) | 1 (4.76%) | | |
| No | 22 (95.65%) | 20 (95.24%) | | |

ETE Extrathyroidal extension, LNM Lymph node metastasis, LLNM Lateral neck lymph node metastasis

Table 4 Multi-factor assignment table

| Variant | Assignment |
|-------------------|-------------|
| ETE | Yes=1, No=0 |
| LNM | Yes=1, No=0 |
| Multifocality | Yes=1, No=0 |
| BRAF Mutation | Yes=1, No=0 |
| RET Rearrangement | Yes=1, No=0 |

ETE Extrathyroidal extension, LNM Lymph node metastasis

rearrangement positivity were subjected to multifactorial COX regression analysis. The results of the analysis showed that TSH, multifocal, LNM, combined Hashimoto, and RET rearrangement had no effect on post-operative recurrence of PTC. While ETE, higher risk of recurrence stratification ($P < 0.05$) had an effect on recurrence (Table 8).

Discussion

In the available studies [2], the prevalence of Hashimoto’s thyroiditis (HT) is increased in thyroid cancer. The presence of homologous HT tends to suggest an association with less aggressive papillary thyroid cancer (PTC), such as lower TNM stage, lower likelihood of centralized LNM, and less peritumoral and vascular infiltration. This suggests a better prognosis, but the association of the coexistence of the two with recurrence and disease-free survival is not conclusive. Recurrence of thyroid cancer can include local recurrence, regional recurrence, and distant metastasis [18]. The results of this study suggest that the rate of central or lateral lymph node recurrence after thyroid surgery is much higher than that of residual thyroid body recurrence (80.95% versus 28.57%). In other words, regional lymph node recurrence predominates in

the recurrence after thyroid surgery. Surgery is the first-line treatment option for thyroid cancer, both for initial diagnosis and recurrence. In our study, we found that PTC patients with combined HT were younger and had higher preoperative TgAb indexes. They also had less ETE, as suggested by postoperative pathology, and more multifocal tumors (defined as two or more anatomically independent foci in the thyroid, including unilateral or bilateral glandular lobe onset [19]. They also had lower LNM, which is consistent with previous studies [20–22] that suggest clinical features such as younger age, less ETE, and negative lymph nodes tend to suggest a better prognosis. Multifocality was also more common in PTC with combined HT in this study, which is consistent with clinical reports [23, 24]. TgAb, a clinical diagnostic indicator of HT, is considered an independent risk factor for the occurrence of LNM in HT combined with PTC [25]. This is thought to be related to the fact that the gene antigenic epitopes of thyroid carcinoma and HT can be recognized by TgAb, thus inducing tumor progression. There was no significant difference between the two groups of patients in terms of maximum tumor diameter. A comprehensive review of the literature [26] suggests that a tumor diameter of >1 cm is a risk factor for the development of central regional LNM in PTC with combined HT, but none of the studies were able to determine a specific cut-off value for tumor diameter. HT occurs more often in women, which may be related to estrogens and progestins [27]. One study suggested that men with PTC with combined HT had a lower incidence but were more likely to develop cervical lymph node metastasis [23]. However, in this study, we did not find a statistically significant gender difference between the two groups. This may be related to a selective bias in the experimental enrollment population.

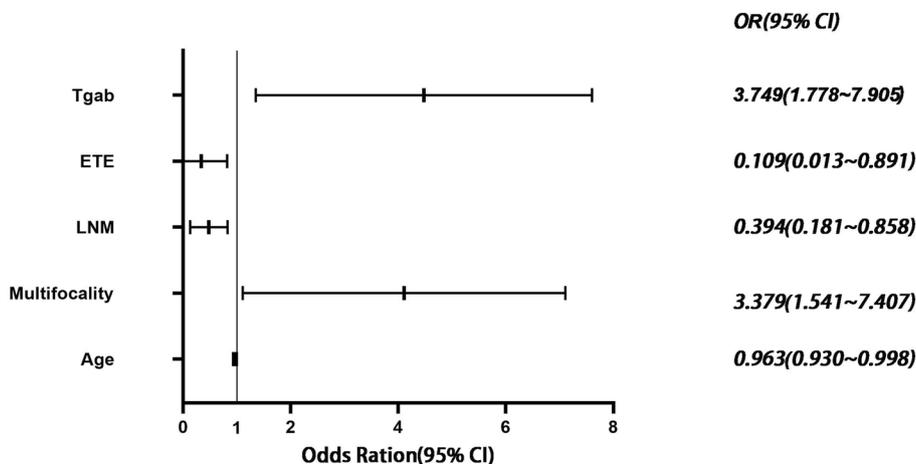


Fig. 2 Binary Logistic Regression Analysis of Independent Risk Factors Causing HT Combined with PTC

Table 5 Initial surgical approach and recurrence site

| Initial Surgical Approach | Cases | Postoperative Recurrence Cases | Recurrence Site | | |
|--|-------|--------------------------------|------------------|------------------------|---------------------------------|
| | | | Residual Thyroid | Lymph nodes Of zone VI | lymph nodes of Lateral cervical |
| Unilateral thyroidectomy+ lymph node dissection in zone VI | 76 | 7 | 6 | 3 | 2 |
| Unilateral thyroidectomy+ lymph node dissection in Unilateral Lateral cervical | 11 | - | - | - | - |
| Total thyroidectomy+ lymph node dissection in zone VI | 117 | 14 | - | 3 | 14 |
| Total thyroidectomy+ lymph node dissection in Unilateral Lateral cervical | 41 | - | - | - | - |
| Total thyroidectomy+ lymph node dissection in Bilateral Lateral cervical | 4 | - | - | - | - |

Table 6 Univariate cox regression analysis of factors influencing recurrence in patients in the HT group

| Characteristics | β | SE | Wald | P | HR | HR (95% CI) |
|-----------------------|---------|--------|-------|-------|----------|--------------------|
| Age | 0.853 | 1.414 | 0.363 | 0.547 | 2.346 | 0.147~37.508 |
| Sex | 0.114 | 0.081 | 1.997 | 0.158 | 1.121 | 0.957~1.314 |
| Tumor Diameter (cm) | 1.626 | 0.700 | 5.395 | 0.020 | 5.083 | 1.289~20.043 |
| Multifocal | 4.651 | 6.017 | 0.597 | 0.440 | 104.658 | 0.001~13841512.980 |
| LNМ | 4.953 | 6.337 | 0.611 | 0.434 | 141.601 | 0.001~35098537.790 |
| ETE | 3.749 | 1.414 | 7.029 | 0.008 | 42.497 | 2.658~679.455 |
| Recurrence risk Stage | 6.633 | 10.338 | 0.412 | 0.521 | 759.836 | 0.000~4.787E+11 |
| TSH | -1.294 | 0.924 | 1.962 | 0.161 | 0.274 | 0.045~1.677 |
| BRAF Mutation | -0.115 | 1.414 | 0.007 | 0.935 | 0.891 | 0.056~14.248 |
| RET Rearrangement | 8.712 | 23.055 | 0.143 | 0.706 | 6075.663 | 0.000~2.558E+23 |

ETE Extrathyroidal extension, LNМ Lymph node metastasis

BRAF mutations are now recognized as molecular markers of poor prognosis in PTC, suggesting a poorer outcome for patients. However, in this study, BRAF mutations were not significantly associated with PTC recurrence in patients with combined HT. A previous study [8] found that BRAF mutations were present in patients with HT, but the lower rate of mutations may be linked to the relatively better prognosis of PTC patients with HT. However, another study [28] reported that BRAF mutations are a significant risk factor for LNМ in PTC patients with HT. Additionally, one article [29] proposed that the PTC microenvironment is composed of a mixture of tumor cells, with only a small portion carrying BRAF mutations. This may lead to an underestimation of the frequency of BRAF

mutations in PTC patients with HT, as the presence of wild-type BRAF lymphocytes can dilute the mutant cells and potentially impact the final outcome. In addition to BRAF mutations, RET chromosomal rearrangements also activate the mitogen-activated protein kinase (MAPK) pathway, contributing to tumorigenesis [30], particularly in cases of PTC with HT. Unlike the more common RET mutations found in medullary thyroid carcinoma (MTC) and MEN2, the most prevalent pro-carcinogenic variant of the RET gene in papillary thyroid carcinoma (PTC) is RET rearrangement [31]. This occurrence is complex and has been the subject of scientific research in recent years. Rhoden et al. [32] detected RET rearrangement in both non-tumor follicular cells of Hashimoto's thyroiditis (HT) and

Table 7 Univariate cox regression analysis of factors influencing recurrence in overall patients

| Characteristics | β | SE | Wald | P | HR (95% CI) |
|---|---------|-------|--------|-------|-------------------------|
| Age | 0.182 | 0.469 | 0.150 | 0.698 | 1.200 (0.478~3.009) |
| Sex | 0.034 | 0.019 | 3.206 | 0.073 | 1.035 (0.997~1.074) |
| Tumor Diameter (cm) | 0.134 | 0.255 | 0.274 | 0.601 | 1.143 (0.693~1.885) |
| Multifocal | 1.615 | 0.469 | 11.858 | 0.001 | 5.027 (2.005~12.604) |
| LNM | 1.780 | 0.627 | 8.058 | 0.005 | 5.927 (1.735~20.250) |
| ETE | 3.159 | 0.518 | 37.248 | 0.000 | 23.554 (8.540~64.967) |
| Recurrence risk Stage | | | | | |
| 1 | | | 35.364 | 0.000 | |
| 2 | 2.955 | 0.659 | 20.140 | 0.000 | 19.208 (5.284~69.826) |
| 3 | 4.117 | 0.693 | 35.326 | 0.000 | 61.352 (15.786~238.448) |
| TSH | 0.038 | 0.010 | 14.977 | 0.000 | 1.039 (1.019~1.059) |
| Coexistence with HT | -0.644 | 0.745 | 0.746 | 0.388 | 0.525 (0.122~2.264) |
| BRAF Mutation | 0.691 | 0.560 | 1.523 | 0.217 | 1.995 (0.666~5.977) |
| RET Rearrangement | 1.248 | 0.562 | 4.928 | 0.026 | 3.483 (1.157~10.484) |
| RET Rearrangement * Coexistence with HT | 1.608 | 0.747 | 4.631 | 0.031 | 4.991 (1.154~21.585) |
| BRAF Mutation * Coexistence with HT | -0.659 | 1.026 | 0.412 | 0.521 | 0.518 (0.069~3.866) |
| BRAF Mutation * RET Rearrangement | 1.755 | 1.032 | 2.893 | 0.089 | 5.783 (0.766~43.682) |

ETE Extrathyroidal extension, LNM Lymph node metastasis, HT Hashimoto's thyroiditis

Table 8 Multifactorial cox regression analysis of factors influencing recurrence in overall patients

| Factors | β | SE | Wald | P | HR | HR (95% CI) |
|-----------------------|---------|-------|--------|-------|--------|--------------|
| ETE | 2.003 | 0.597 | 11.265 | 0.001 | 7.410 | 2.301~23.864 |
| Recurrence Risk Stage | | | | | | |
| 1 | - | - | 8.818 | 0.012 | - | - |
| 2 | 2.096 | 0.853 | 6.036 | 0.014 | 8.134 | 1.528~43.301 |
| 3 | 2.682 | 0.910 | 8.681 | 0.003 | 14.610 | 2.454~86.972 |

ETE Extrathyroidal extension

PTC, suggesting a potential shared molecular mechanism between the two. Anna [15] et al. concluded that while RET rearrangement has been reported in PTC, it is a relatively rare event in HT. It has been proposed that HT may induce RET rearrangement, essentially turning it into a gene for thyroid cancer, due to the body's immune response to thyroid cancer antigens. The pathology of HT is characterized by the gradual replacement of thyroid gland epithelial cells with mononucleated cells, leading to suggestions that HT may be considered a precancerous lesion of PTC [33]. In addition, RET rearrangement can activate the downstream signaling system, leading to increased inflammation and creating a microenvironment that promotes tumorigenesis. The HT group in this study had a lower BRAF mutation rate and a higher RET rearrangement rate.

The recurrence rate of PTC patients with combined HT was 4.55%, which was lower than the NHT group (9.27%) and the 8%–28% reported in previous clinical studies [34]. Although the difference in recurrence rate was not statistically significant, further subgroup analysis revealed that the rate of LNM and recurrence was higher in RET-positive patients in the HT group ($P < 0.05$). One-way cox regression analysis suggested that RET rearrangement positivity and its interaction with Hashimoto's effect may be potential risk factors for recurrence. However, after multifactorial regression analysis, only ETE and risk stratification for recurrence were retained as independent predictors of recurrence. Therefore, the role of RET rearrangement in recurrence needs to be further explored in future studies.

The present study has several limitations that should be noted. Firstly, while NGS allows for a more comprehensive detection of genes, the overall sample size is limited and is derived from a single center. Additionally, this study is retrospective in nature, which may be limited by the completeness and accuracy of the clinical case records. Furthermore, the sample population is restricted due to the single center, which could be addressed by expanding the sample size, conducting a multicenter prospective study, and incorporating more imaging data.

In summary, several indicators, including multifocal tumors, TgAb levels, younger age, and negative lymph nodes, can aid in the preoperative diagnosis of HT-combined PTC. Additionally, BRAF mutations are less common in HT-combined PTC and do not significantly impact recurrence. However, the presence of RET rearrangement is associated with a higher recurrence rate in HT-combined PTC, highlighting the importance for clinicians to carefully consider the extent of cleared lymph nodes and resected glands during surgery and to closely monitor patients postoperatively.

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Authors' contributions

All authors contributed to the study conception and design. Material preparation, data collection and analysis were performed by Z. The first draft of the manuscript was written by Z and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

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Data availability

No datasets were generated or analysed during the current study.

Declarations

Ethics approval and consent to participate

This study was performed in line with the principles of the Declaration of Helsinki. Approval was granted by the Ethics Committee of the First Affiliated Hospital of Anhui Medical University (Quick-PJ- 2022-13 -44).

Consent for publication

The Authors agree to publication in the Journal *Diagnostic Pathology*.

Competing interests

The authors declare no competing interests.

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