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Utilizing Immunoglobulin G4 Immunohistochemistry for Risk Stratification in Patients with Papillary Thyroid Carcinoma Associated with Hashimoto Thyroiditis

Faridul Haq[1,2,*], Gyeongsin Park[3,*], Sora Jeon[2], Mitsuyoshi Hirokawa[4], Chan Kwon Jung[1,2,3]

[1]Department of Biomedicine and Health Sciences, The Catholic University of Korea; [2]Cancer Research Institute, [3]Department of Hospital Pathology, College of Medicine, The Catholic University of Korea, Seoul, Korea; [4]Department of Diagnostic Pathology and Cytology, Kuma Hospital, Kobe, Japan

Background: Hashimoto thyroiditis (HT) is suspected to correlate with papillary thyroid carcinoma (PTC) development. While some HT cases exhibit histologic features of immunoglobulin G4 (IgG4)-related disease, the relationship of HT with PTC progression remains unestablished.

Methods: This cross-sectional study included 426 adult patients with PTC (≥1 cm) undergoing thyroidectomy at an academic thyroid center. HT was identified based on its typical histologic features. IgG4 and IgG immunohistochemistry were performed. Whole-slide images of immunostained slides were digitalized. Positive plasma cells per 2 mm^2 were counted using QuPath and a pre-trained deep learning model. The primary outcome was tumor structural recurrence post-surgery.

Results: Among the 426 PTC patients, 79 were diagnosed with HT. With a 40% IgG4 positive/IgG plasma cell ratio as the threshold for diagnosing IgG4-related disease, a cutoff value of >150 IgG4 positive plasma cells per 2 mm^2 was established. According to this criterion, 53% (43/79) of HT patients were classified as IgG4-related. The IgG4-related HT subgroup presented a more advanced cancer stage than the IgG4-non-related HT group (P=0.038). The median observation period was 109 months (range, 6 to 142). Initial assessment revealed 43 recurrence cases. Recurrence-free survival periods showed significant (P=0.023) differences, with patients with IgG4 non-related HT showing the longest period, followed by patients without HT and those with IgG4-related HT.

Conclusion: This study effectively stratified recurrence risk in PTC patients based on HT status and IgG4-related subtypes. These findings may contribute to better-informed treatment decisions and patient care strategies.

Keywords: Hashimoto disease; Cross-sectional studies; Thyroid neoplasms; Thyroid cancer, papillary; Immunoglobulin G4-related disease; Immunohistochemistry; Deep learning; Patient care

INTRODUCTION

Hashimoto thyroiditis (HT) is a chronic autoimmune disorder that affects the thyroid gland. It is the leading cause of hypothyroidism in iodine-sufficient areas of the world [1]. About 20% to 30% of patients with hypothyroidism suffer from HT, whose

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Corresponding author: Chan Kwon Jung
Department of Pathology, Seoul St. Mary's Hospital, College of Medicine, The Catholic University of Korea, 222 Banpo-daero, Seocho-gu, Seoul 06591, Korea
Tel: +82-2-2258-1622, **Fax:** +82-2-2258-1627, **E-mail:** ckjung@catholic.ac.kr

*These authors contributed equally to this work.

cause is thought to be a combination of genetic susceptibility and environmental factors that can lead to loss of immunological tolerance with consequent autoimmune attack to the thyroid tissue and appearance of the disease [1]. The disease is characterized by the production of autoantibodies against thyroid antigens, which can lead to lymphocytic infiltration, progressive destruction of thyroid follicles, and oncocytic metaplasia of follicular cells, ultimately reducing thyroid hormone production [2]. HT typically affects more women than men. It is most commonly diagnosed in middle-aged individuals [3]. Diagnosis of HT involves a combination of clinical evaluation, laboratory testing, and imaging studies [1]. Definitive diagnosis of HT is based on characteristic histopathological findings. The most common laboratory findings of HT include elevated levels of thyroid autoantibodies and reduced levels of thyroid hormones. Imaging studies such as ultrasound can reveal a heterogeneous thyroid gland with reduced echogenicity [4].

The association between HT and papillary thyroid carcinoma (PTC) has been well-documented in the scientific literature [5-7]. While the question of a causal link remains a subject of ongoing debate, both conditions exhibit common epidemiological characteristics such as relatively high prevalence, a propensity to affect females, and a tendency to occur in iodine-sufficient regions [5-7]. Notably, in individuals diagnosed with HT, PTC appears with greater frequency but tends to manifest in a less aggressive form [5,7,8]. Nonetheless, the literature did not uniformly support the notion that HT could exert a protective effect against the progression of PTC. Several studies have found no significant correlation between HT and the progression or prognosis of PTC in patients [7].

HT is a heterogeneous disease that can be categorized into two subtypes based on the density of immunoglobulin G4 (IgG4)-positive plasma cells in the thyroid tissue: IgG4-related and non-IgG4-related [9-11]. The IgG4-related subtype exhibits histopathological hallmarks of systemic IgG4-related disease within the thyroid gland. Histological evaluation is crucial for its diagnosis, typically revealing a high concentration of lymphoplasmacytic cells, unique storiform fibrosis, and obliterative phlebitis [12]. However, IgG4-related HT generally does not present with storiform fibrosis, obliterative phlebitis, or systemic manifestations [13]. Various cutoff values have been suggested for IgG4-positive plasma cell counts [13]. Traditionally, IgG4+ and IgG+ cells have been counted using printed photographs taken at a 40× objective lens magnification. However, the advent of digital pathology mandates a shift away from high-power field (HPF) as the standard unit for cell counting. Instead, a more globally standardized unit, square millimeters, should be employed [14]. This shift aligns well with quantitative capabilities of digital image analysis, offering a more standardized and reproducible approach to cell counting. The IgG4+/IgG+ ratio serves as an additional diagnostic criterion for IgG4-related disease, with proposed cutoffs ranging from 0.3 to 0.5 [13]. In most documented cases of IgG4-related disease, the cutoff of IgG4-positive to IgG-positive plasma cell ratio is 40% [15]. This ratio is a more reliable diagnostic tool than merely counting IgG4-positive plasma cells in IgG4-related disease. However, a challenge in utilizing this ratio is a high background staining observed in IgG immunostaining, which can hinder a precise calculation of the IgG4+/IgG+ ratio. In this regard, our study aimed to establish clinically relevant IgG4+ cutoff values using mm^2 as the unit of measurement, specifically for patients with PTC.

This study primarily sought to establish a diagnostic methodology for clinical utility of IgG4-related HT using digital pathology and image analysis. Additionally, we aimed to stratify patients with PTC prognostically based on the presence and IgG4-related status of HT in accordance with our newly developed diagnostic criteria.

METHODS

Study cohort

This study was performed in line with the principles of the Declaration of Helsinki. It was approved by the Institutional Review Board of Seoul St. Mary's Hospital (KC16SISI0104), College of Medicine, The Catholic University of Korea. Informed consent was waived by the board. The cohort comprised 426 patients who underwent surgical intervention for PTC between January 2008 and December 2010. This research builds upon the same patient group featured in our earlier studies [16-20]. However, patient counts differed due to specimen availability and an unavoidable tissue loss during sectioning and staining processes involving archival tissue blocks. Clinical follow-up data were updated through March 2022.

The different subtypes of PTC were identified based on the 5th edition of the World Health Organization's Classification of Endocrine and Neuroendocrine Tumors [21,22]. The subtypes include classic PTC (n=376), encapsulated classic PTC (n=24), infiltrative follicular PTC (n=10), invasive encapsulated follicular PTC (n=4), tall cell PTC (n=17), and other subtypes (n=19), such as oncocytic PTC (n=9), Warthin-like PTC (n=7), hobnail PTC (n=2), and diffuse sclerosing PTC (n=1). Cancer

staging was determined according to the 8th edition of the American Joint Committee on Cancer (AJCC) staging manual [23].

Structural recurrence was defined as detection of recurrent disease that was identifiable on imaging studies or through pathologic confirmation. This included, but not limited to, identification of malignant tissue within the thyroidectomy bed, regional lymphatic structures, and any distal metastatic sites. For precise assessment of structural recurrence-free survival (RFS), the study design necessitated exclusion of a subset of eight patients who presented with either regional or distant metastatic disease prior to their thyroidectomy or who developed such manifestations within a 6-month window following the surgical procedure. This exclusion criterion was applied to distinguish between persistent primary disease and actual disease recurrence, thereby refining the prognostic utility of the structural RFS metric.

Histomorphological analysis

Histomorphological evaluation of HT entailed microscopic scrutiny of thyroid tissue to identify salient histological hallmarks characteristic of this autoimmune condition. The thyroid gland of patients with HT displayed diffuse lymphoplasmacytic infiltration, lymphoid follicles, and oncocytic metaplasia of follicular cells (Fig. 1A).

Immunohistochemistry

Immunohistochemical staining was conducted for IgG and IgG4 utilizing an automated Ventana BenchMark ULTRA Staining System (Roche Diagnostics, Tucson, AZ, USA). From each case, a representative formalin-fixed, paraffin-embedded block was sliced into 4-μm-thick sections. Antigen retrieval was performed for 48 minutes using a Ventana Benchmark CC1 standard program. Subsequently, tissue sections were incubated with an anti-IgG4 mouse monoclonal antibody (clone MRQ-44, prediluted, Roche Diagnostics) at a temperature of 37°C for 16 minutes and an anti-IgG rabbit polyclonal antibody (prediluted, Roche Diagnostics) at room temperature for 12 minutes. The immunoreaction was visualized employing a Ventana OptiView DAB Immunohistochemical Detection Kit (with OptiView HQ Linker for 8 minutes and OptiView HRP Multimer for another 8 minutes) and an OptiView Amplification Kit. Counterstaining was performed using Ventana Hematoxylin II for 4 minutes, followed treatment with a bluing reagent for 4 minutes (Fig. 1B, C). Tonsil tissue served as a positive control for the procedure.

Digital image analysis

All slides were digitally scanned at a 20× magnification,

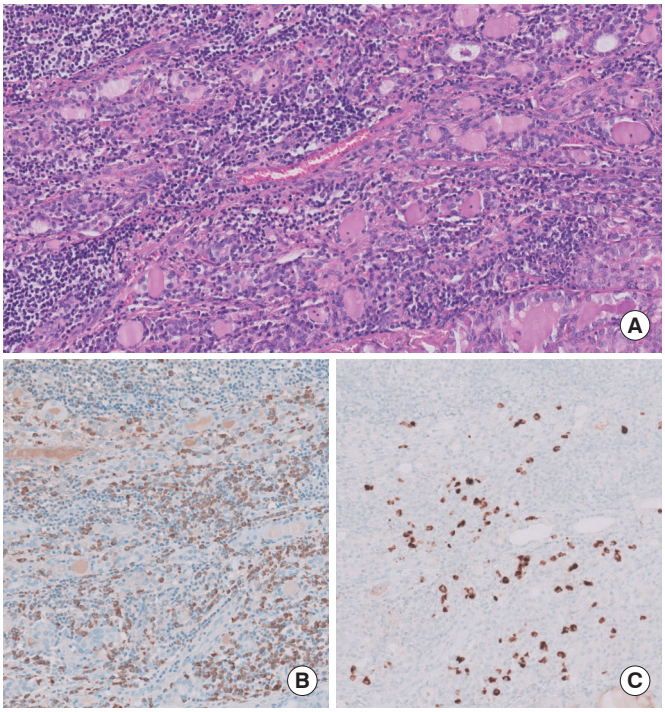

Fig. 1. Histopathological and immunohistochemical features of immunoglobulin G4 (IgG4)-related Hashimoto thyroiditis. (A) Microscopic examination reveals a dense infiltration of lymphocytes and plasma cells, leading to the loss and damage of thyroid follicles. The stromal background appears fibrotic. Follicular cells display oncocytic metaplasia, characterized by plump eosinophilic cytoplasm, enlarged nuclei, and clear chromatin (hematoxylin and eosin stain, digital zoom ×20). (B) Immunohistochemical staining shows a high density of IgG-positive plasma cells (immunohistochemistry, digital zoom ×20). (C) A significant presence of IgG4-positive plasma cells is also noted (immunohistochemistry, digital zoom ×20).

achieving a resolution of 0.46 μm per pixel, using a Hamamatsu NanoZoomer S360 Digital Slide Scanner (Hamamatsu Photonics, Hamamatsu, Japan). For digital image analysis, scanned images were imported into QuPath version 0.4.3 (https://github.com/qupath/qupath/releases/), an open-source software platform specifically designed for whole-slide image (WSI) analysis [24].

To pinpoint "hotspot" areas rich in IgG and IgG4-positive plasma cells, a semi-automated methodology was adopted. The software generated measurement heat maps by visualizing optical density of immunostained plasma cells. Utilizing these heat maps, densities of IgG and IgG4-positive plasma cells were quantitatively assessed. Regions with the highest density of positively stained cells, termed "hotspots," were manually outlined. Meticulous adjustments were executed to omit areas marred by artifacts or false-positive staining. These heat maps

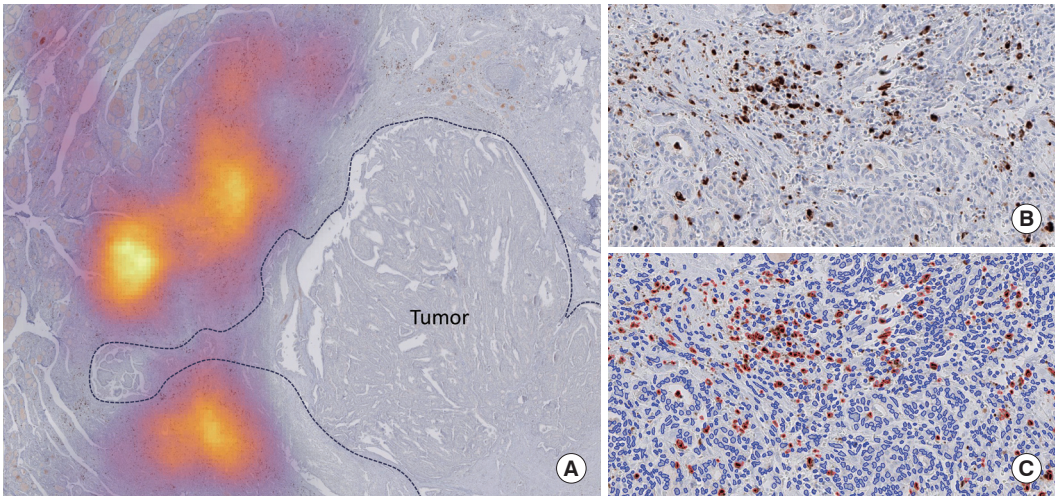

Fig. 2. An example of hotspot identification for immunoglobulin G4 (IgG4)-positive plasma cells in non-tumor regions using QuPath software. (A) A measurement heat map displaying the area with the highest density of IgG4-positive plasma cells in non-tumor area (immunohistochemistry, digital zoom ×5). A dotted line demarcates the tumor region. (B) A magnified view of the targeted area, highlighting IgG4 immunostaining within the identified hotspot (immunohistochemistry, digital zoom ×20). (C) A processed analytical image depicting differentiation of cells, with negative cells marked in blue and positive cells highlighted in red (immunohistochemistry, digital zoom ×20).

were individually fine-tuned for each WSI to accurately identify and annotate regions with the maximal density of IgG and IgG4-positive plasma cells. Areas susceptible to artifacts, which could result in an erroneous identification of hotspots, were manually excluded from analysis. Plasma cells within a designated area of 2 mm^2 were then counted (Fig. 2).

Statistical analysis

Relationships of clinicopathologic features with IgG4-related status of HT were analyzed using a Pearson chi-square or Fisher's exact for categorical variables as appropriate. The Spearman's rank-based correlation coefficient (r) was employed to assess the degree of association between two continuous variables. The Kaplan-Meier method was used to analyze RFS and the long-rank test was used to compare groups. RFS was measured from the date of the initial surgery until the identification of structural disease recurrence or until the last follow-up for patients without any signs of recurrence. To determine prognostic significance of clinical features, pathological features, and IgG4 status, Cox proportional hazards regression models were used for both univariate and multivariate analyses. For the multivariate analysis, potential confounders were adjusted for and independent factors that could predict RFS were identified. We calculated hazard ratios and 95% confidence intervals to measure the risk associated with individual variables. All statistical values were calculated using the statistical software program SPSS version 21.0 (IBM Corp., Armonk, NY, USA) and GraphPad Prism version 6.07 (GraphPad, La Jolla, CA, USA). A $P<0.05$ was considered statistically significant.

RESULTS

Clinical characteristics

This study encompassed 426 patients with PTC who were stratified into two cohorts: those without HT ($n=347$) and those with HT ($n=79$) (Table 1). Age distribution between cohorts did not display statistically significant difference ($P=0.457$), with the majority of both cohorts being younger than 55 years (69.2% in those without HT and 73.4% in those with HT). Sex distribution was significantly skewed towards females, particularly within the HT group (96.2% vs. 71.8% in the non-HT group, $P<0.000$).

Histologic subtype analysis revealed a predominance of the classic subtype in both patient groups (Table 1). However, the classic subtype was less prevalent in the HT group than in the non-HT group (81.0% vs. 89.9%, $P<0.001$). Other subtypes, including follicular ($n=14$) tall cell ($n=17$) and others (one solid, two hobnail, two Warthin-like, and eight oncocytic PTCs) were comparably distributed between the two groups. The prevalence of histologically aggressive subtypes did not significantly differ between cohorts ($P=0.764$). HT status was not associated with other pathologic parameters or $BRAF^{V600E}$.

Table 1. Clinicopathologic Features of 426 Patients with Papillary Thyroid Carcinoma Stratified by Hashimoto Thyroiditis

Characteristic	Patients without HT (*n*=347)	Patients with HT (*n*=79)	*P* value
Age, yr			0.457
<55	240 (69.2)	58 (73.4)	
≥55	107 (30.8)	21 (26.6)	
Sex			<0.000
Female	249 (71.8)	76 (96.2)	
Male	98 (28.2)	3 (3.8)	
Histologic subtype			<0.001
Classic	312 (89.9)	64 (81.0)	
Follicular	13 (3.7)	1 (1.3)	
Tall cell	14 (4.0)	3 (3.8)	
Warthin-like	1 (0.3)	5 (6.3)	
Other	7 (2.0)	6 (7.6)	
Histologically aggressive subtype			0.764
Aggressive subtype[a]	15 (4.3)	4 (5.1)	
Non-aggressive subtype	332 (95.7)	75 (94.9)	
Multifocality			0.961
Present	146 (42.1)	33 (41.8)	
Absent	201 (57.9)	46 (58.2)	
Extrathyroidal extension			0.394
Present	262 (75.5)	56 (70.9)	
Absent	85 (24.5)	23 (29.1)	
Gross extrathyroidal extension			0.152
Present	73 (21.0)	11 (13.9)	
Absent	274 (79.0)	68 (86.1)	
Pathologic (p) T category			0.112
pT1–2	271 (78.1)	68 (86.1)	
pT3–4	76 (21.9)	11 (13.9)	
pN category			0.486
pN0	126 (36.3)	32 (40.5)	
pN1	221 (63.7)	47 (59.5)	
AJCC stage, 8th edition			0.139
Stage I–II	334 (96.3)	79 (100)	
Stage III–IV	13 (3.7)	0	
Structural recurrence[b]			0.958
Present	35 (10.3)	8 (10.1)	
Absent	304 (89.7)	71 (89.9)	
BRAF^{V600E}			0.378
Present	295 (85.0)	64 (81.0)	
Absent	52 (15.0)	15 (19.0)	

Values are expressed as number (%).
HT, Hashimoto thyroiditis; AJCC, American Joint Committee on Cancer.
[a]Aggressive subtype includes tall cell (*n*=17) and hobnail (*n*=2) subtypes; [b]For the assessment of structural recurrence, we excluded eight patients who manifested regional or distant metastasis prior to thyroidectomy or within a 6-month postoperative interval.

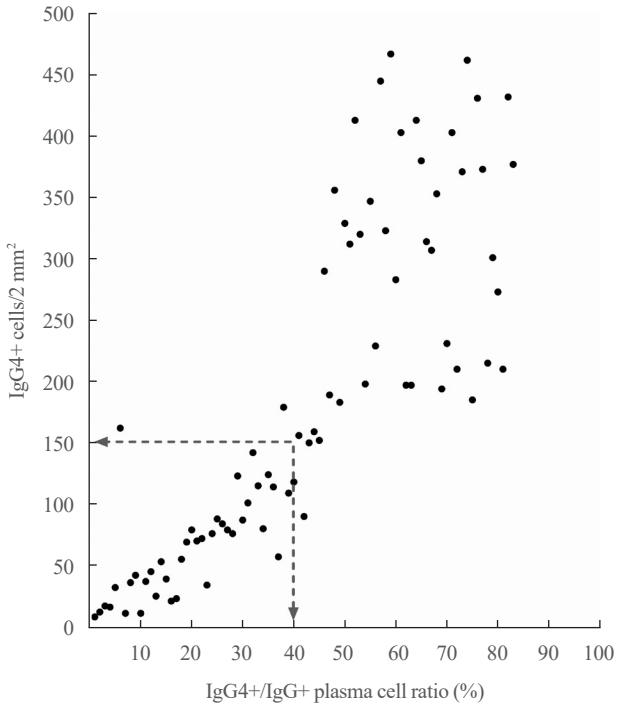

Fig. 3. Relationship between absolute numbers of immunoglobulin G4 (IgG4)-positive (+) cells and IgG4+/IgG+ plasma cell ratio. A 40% IgG4+/IgG+ plasma cell ratio aligns with 150 IgG4+ plasma cells per 2 mm^2.

Determining the threshold value of IgG4 positive plasma cells

We conducted a detailed quantification of IgG and IgG4-positive plasma cells within a 2 mm^2 area of histological hotspots using QuPath software. A direct correlation was observed between the absolute number of IgG4-positive (+) cells and the IgG4+/IgG+ plasma cell ratio (Fig. 3). Applying a 40% IgG4+/IgG+ plasma cell ratio as the criterion for IgG4-related disease, we identified a threshold of over 150 IgG4+ plasma cells per 2 mm^2. This threshold allowed for the classification of 53% (42 of 79) of HT cases as IgG4-related.

Correlation of IgG4 positive cell density in intratumoral and extratumoral tissues

We counted IgG4 positive cells per 2 mm^2 within intratumoral and adjacent non-tumor tissue areas. To determine the correlation between these two microenvironments, we used a scatter plot and a regression analysis. Results showed a weak positive linear relationship with a *r* of 0.187 (Fig. 4). This suggested that there was a slight tendency for an increase in IgG4 positive cell density in both intratumoral and non-tumoral areas, although this association was not strong. The scatter plot revealed a large

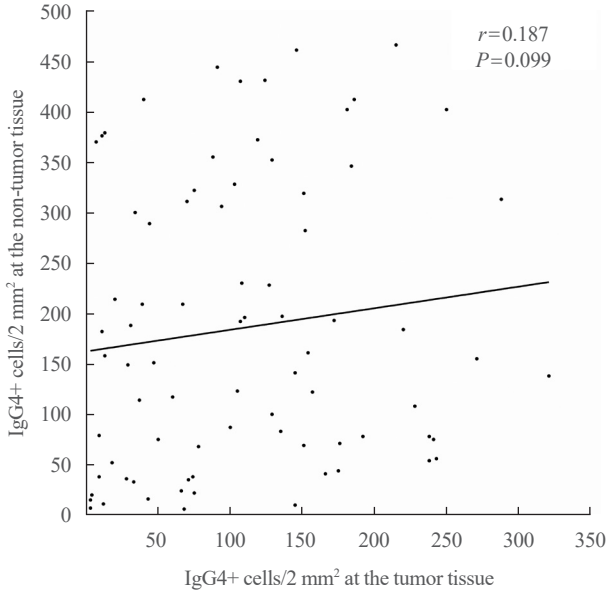

Fig. 4. Analysis of correlation of immunoglobulin G4 (IgG4) positive cell density in intratumoral and extratumoral tissues shows a weak positive linear correlation with a correlation coefficient (*r*) of 0.187.

spread of data points, further confirming the weak correlation (Fig. 4). Therefore, the density of IgG4 positive cells within tumor tissues cannot be used as a reliable predictor for the density in adjacent non-tumor tissues.

Subclassification of patients with HT according to IgG4 count

Patients were further classified according to the threshold of 150 IgG4+ plasma cells per 2 mm^2 into two groups: IgG4-unrelated HT (n=37) and IgG4-related HT (n=42) (Table 2). The analysis did not reveal any significant differences in pathologic T category (P=0.269) or N category (P=0.355). However, patients with IgG4-related HT had a significantly higher incidence of advanced disease than patients with IgG4-unrelated HT (AJCC stage III–IV: 40% vs. 19%, P=0.038). Moreover, the IgG4-related HT group showed a significantly higher rate of structural recurrence than the IgG4-unrelated HT group (19% vs. 0%, P=0.005). In addition, *BRAF*^{V600E} variant was detected more frequently in the IgG4-related group than in the IgG4-unrelated HT group, although the difference between the two was not statistically significant (88% vs. 73%, P=0.087).

Table 2. Differences of Clinicopathological Features between IgG4-Related and IgG4-Unrelated Papillary Thyroid Carcinomas in 79 Patients with Hashimoto Thyroiditis

Characteristic	IgG4-unrelated HT (n=37)	IgG4-related HT (n=42)	P value
Age, yr			0.483
<55	25 (68)	33 (79)	
≥55	12 (32)	9 (21)	
Sex			0.597
Female	35 (95)	41 (98)	
Male	2 (5)	1 (2)	
Histologic subtype			0.254
Classic	28 (76)	36 (86)	
Follicular	1 (3)	0	
Tall cell	1 (3)	2 (5)	
Warthin-like	3 (8)	2 (5)	
Other	4 (11)	2 (5)	
Histologically aggressive subtype			0.896
Aggressive subtype	2 (5)	2 (5)	
Non-aggressive subtype	35 (95)	40 (95)	
Extrathyroidal extension			0.269
Present	24 (65)	32 (76)	
Absent	13 (35)	10 (24)	
Gross extrathyroidal extension			0.453
Present	4 (11)	7 (17)	
Absent	33 (89)	35 (83)	
Pathologic (p) T category			0.269
pT1–2	13 (35)	10 (24)	
pT3–4	24 (65)	32 (76)	
pN category			0.355
pN0	17 (46)	15 (36)	
pN1	20 (54)	27 (64)	
AJCC stage, 8th edition			0.038
Stage I–II	30 (81)	25 (60)	
Stage III–IV	7 (19)	17 (40)	
Structural recurrence			0.005
Present	0	8 (19)	
Absent	37 (100)	34 (81)	
BRAF^{V600E}			0.087
Present	27 (73)	37 (88)	
Absent	10 (27)	5 (12)	

Values are expressed as number (%).
IgG4, immunoglobulin G4; HT, Hashimoto thyroiditis; AJCC, American Joint Committee on Cancer.

Recurrence-free survival

In the cohort of 426 patients, who underwent surgery for PTC, eight were excluded due to the presence of distant metastasis at the time of surgery or a follow-up period of less than 6 months. Among the remaining 418 patients, the median followed-up period was 109 months (range, 6 to 142) and the structural recur-

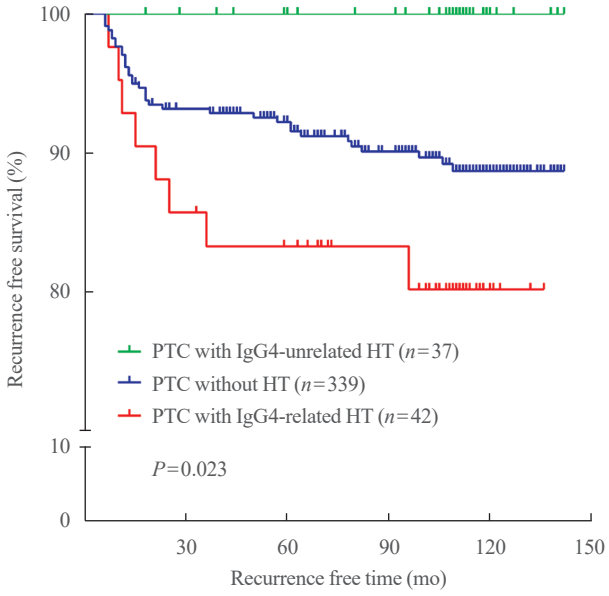

Fig. 5. Kaplan-Meier survival analysis showing recurrence-free survival in patients with papillary thyroid carcinoma (PTC) stratified by the presence of Hashimoto thyroiditis (HT) and immunoglobulin G4 (IgG4)-related subtype.

rence, rate was 10.3% (43 of 418 patients).

Using Kaplan-Meier plot and log-rank survival analysis, RFS was analyzed amongst patients with PTC based on the presence and IgG4-related status of HT. We included three groups: PTC free HT (n=339), PTC with IgG4-unrelated HT (n=37), and PTC with IgG4-related HT (n=42). Results of analysis showed significantly different rates of RFS among the three groups (P=0.023) (Fig. 5). Patients with IgG4-unrelated HT experienced no recurrences, whereas those with IgG4-related HT demonstrated the poorest RFS. PTC patients without HT exhibited an intermediate RFS. These results offer supportive evidence on the adverse effect that IgG4-related status of HT can impose on PTC prognosis.

In multivariate analysis, lymph node metastasis, gross extrathyroidal extension, and pT category were independently associated with a diminished RFS (P=0.001, P=0.039, P=0.002, respectively), as shown in Table 3. The presence of IgG4-related HT was also associated with a worse RFS, with a hazard ratio of 2.227 (95% confidence interval, 1.028 to 4.822; P=0.042).

Table 3. Multivariate Cox Regression Analysis of Prognostic Variables for Recurrence-Free Survival in 426 Patients with Papillary Thyroid Carcinoma

Variable	Hazard ratio	95% CI	*P* value
Age, yr			0.223
<55	1	Reference	
≥55	0.600	0.264–1.363	
Sex			0.772
Female	1	Reference	
Male	1.113	0.541–2.288	
Histologic subtype			0.493
Non-aggressive	1	Reference	
Aggressive	1.517	0.460–4.996	
Gross extrathyroidal extension			0.039
Absent	1	Reference	
Present	4.813	1.086–21.339	
Pathologic (p) T category			0.002
pT1–2	1	Reference	
pT3–4	11.910	2.414–58.756	
pN			0.001
pN0	1	Reference	
pN1	7.380	2.265–24.051	
IgG4-related HT			0.042
Absent	1	Reference	
Present	2.227	1.028–4.822	

CI, confidence interval; IgG4, immunoglobulin G4; HT, Hashimoto thyroiditis.

DISCUSSION

Our study provides a new perspective on the prognostic landscape of PTC when concurrent HT is present, with an emphasis on the usefulness of IgG4 immunohistochemistry as a tool for risk stratification. The present study highlights the significance of determining a specific threshold of IgG4 count (150 IgG-positive cells per 2 mm^2) as a predictive marker for PTC behavior in the context of HT. Our findings indicate that exceeding this threshold is associated with advanced cancer stage and higher structural recurrence rate of the disease, suggesting a more aggressive PTC phenotype. Besides being a potential indicator of disease severity, the identified threshold can also help clinicians refine prognostic accuracy while managing PTC in the presence of HT.

A Japanese research team has proposed thyroid-specific cut-off values for the diagnosis of IgG4-related thyroiditis, recommending more than 20 IgG4-positive plasma cells per HPF and an IgG4-to-IgG plasma cell ratio exceeding 30% [25]. As the disease progresses into later stages characterized by increasing

Table 4. International Studies on the Prevalence of IgG4-Related Cases among Patients with Hashimoto Thyroiditis

Study	Country	Institution[a]	Study year	Reasons for thyroidectomy in patients with HT (*n*)	IgG4 count	IgG4/IgG ratio	Prevalence
Li et al. (2020) [31]	Japan, China	Kuma Hospital, Wakayama Medical University, Taishan Medical University	1983–2017	Marked swelling (57), tracheal stenosis (19), pain and tenderness (4), nodular lesion (13), suspected malignant lymphoma (12), suspected PTC (2), unknown (13)	>20/HPF >10/HPF	>30% >40%	33/120 (27.5%) 27/120 (22.5%)
Li et al. (2012) [32]	Japan	Kuma Hospital	1983–2010	Marked swelling (55), tracheal stenosis (19), pain and tenderness (4), nodule (13), suspicious for lymphoma (12), suspicious for PTC (2)	>20/HPF	>30%	28/105 (26.6%)
Li et al. (2010) [33]	Japan	Kuma Hospital	1983–2006	Marked swelling (39), tracheal stenosis (15), suspicious of malignant lymphoma (8), nodular lesion (4), and pain and tenderness (4)	>20/HPF	>30%	19/70 (27%)
Yu et al. (2018) [34]	China	Peking University First Hospital	2009–2016	Marked swelling (14), tracheal compression (4), suspected PTC (70), needle biopsies (5)	>20/HPF	>30%	20/93 (22%)
Yu et al. (2016) [35]	China	Peking University First Hospital	2009–2014	Suspicious for thyroid cancer (64), tracheal compression (2)	>20/HPF	>30%	48/66 (73%)
Zhang et al. (2014) [36]	China	Peking University First Hospital	2009–2012	Suspicious for thyroid cancer (51), tracheal compression (2)	>20/HPF	> 30%	12/53 (23%)
Deshpande et al. (2012) [37]	USA	Massachusetts General Hospital	NA	Histological diagnosis of HT (28), FV of HT (9)	>30/HPF	>40%	4/28 HT (14%) 6/9 FV of HT (67%)
Raess et al. (2015) [38]	USA	Oregon Health & Science University	2010–2013	Clinical HT (23) Malignancy was excluded.	>30/HPF	>30%	8/23 (35%)
Lintusaari et al. (2020) [39]	Finland	Fimlab Laboratories, Tampere	2011–2017	Goiter (37), suspicious for malignancy (37), thyrotoxicosis (3), thyroiditis (1), hyperparathyroidism (1), other (3)	>20/HPF	>30%	13/82 (16%)
Jokisch et al. (2016) [40]	Germany	Institute of Pathology of the Staedtisches Klinikum Munich	2001–2013	Histological diagnosis of HT (191)	>20/HPF	>40%	24/191 (12.6%)
Current study	Korea	Seoul St. Mary's Hospital	2008–2010	PTC	>150/2 mm^2	>40%	42/79 (53%)

IgG4, immunoglobulin G4; HT, Hashimoto thyroiditis; PTC, papillary thyroid carcinoma; HPF, high-power field; NA, not available; FV, fibrosing variant.
[a]These studies conducted by Kuma Hospital and Peking University First Hospital in Japan and China resulted in redundant data due to overlapping patient cohorts.

fibrosis, the number of plasma cells diminishes, making the IgG4/IgG ratio an increasingly valuable diagnostic indicator. However, a 40% IgG4+/IgG+ plasma cell ratio is conventionally accepted as the diagnostic threshold for IgG4-related diseases. It is considered a more robust diagnostic marker than the absolute count of IgG4-positive cells alone [15,26-29]. We adopted the 40% IgG4+/IgG+ plasma cell ratio as a means of identifying IgG4-related HT.

A significant challenge in the application of this ratio is the nonspecific high background staining commonly associated with IgG immunostainings, which can interfere with accurate ratio determinations. Historically, cell counts for IgG4+ and IgG+ have been conducted through analysis of printed photographs at a magnification of 40×. However, with integration of digital pathology, there has been a paradigm shift from using the HPF as a standard unit of measure to a more universally standardized unit of square millimeters (mm^2) [14,30]. Our research sought to delineate IgG4+ cutoff values that could be clinically applicable for PTC patients with HT using mm^2 as the measurement standard. The quantification of 150 IgG4-positive plasma cells per 2 mm^2 area provides valuable insights into the density and infiltration of these cells within examined tissue samples. This observation indicates a significant presence of IgG4-positive plasma cells and highlights their potential role in the pathogenesis or progression of the studied condition.

The prevalence of IgG4-related HT varies depending on different factors, such as geographic location, patient populations, and diagnostic criteria (Table 4). Previous studies have shown a range of prevalence rates due to variations in diagnostic criteria, particularly IgG4 count and IgG4/IgG ratio [31-40]. Studies

conducted in Japan, China, and Korea have demonstrated varying prevalence of IgG4-related HT. Li et al.'s [31] study showed a prevalence ranging from 22.5% to 27.5%, with IgG4 counts of >10/HPF and >20/HPF. In contrast, our study conducted in Korea showed a remarkably higher prevalence of 53%. However, we counted IgG4-positive cells in a larger area (2 mm^2). All patients enrolled in our study had PTC. The prevalence of IgG4-related HT also varied by geographic location and institution. For instance, studies from China and Japan showed a prevalence range of 22% to 73%, illustrating substantial variability even within a relatively homogenous East Asian context. Further, studies conducted in the USA indicated a prevalence of 14% to 35% [37,38], while European studies reported a lower prevalence of 16% in Finland [39] and 12.6% in Germany [40]. These differences might be attributable to variations in patient populations, healthcare practices, and possibly differing environmental or genetic predispositions. The prevalence of IgG4-related HT varies significantly based on reasons for thyroidectomy in patients with HT. For instance, a Chinese study in 2016 found a high prevalence rate (73%) in a population primarily suspected for thyroid cancer [35], while studies involving more general HT populations, such as the USA study [37,38], showed lower rates. This suggests that the context of diagnosis plays a crucial role in determining the prevalence of IgG4-related HT. Therefore, there is a need for standardized diagnostic criteria and a better understanding of the underlying mechanisms of this condition. Future research should aim to reconcile these differences possibly through multinational, multi-institutional studies employing uniform diagnostic criteria to provide a more cohesive understanding of the epidemiology of IgG4-related HT.

Chronic exposure to cancer antigen can trigger the class switch of B lymphocytes to produce IgG4. Elevated IgG4 can interact with cancer bound IgG through its Fc-Fc binding property. It can also engage with Fc receptors on immune effector cells [41]. Through its distinct structural and biological characteristics, heightened IgG4 within the cancer microenvironment facilitates an efficient immune escape for cancer [42]. IgG4 antibodies possess unique immunological properties. They might contribute to classical tolerance mechanisms in autoimmune diseases [41]. These antibodies are present in various diseases, including IgG4-related diseases, IgG4 autoimmune diseases, allergy, cancer, rheumatoid arthritis, and parasite infestation [41]. The presence of IgG4-positive plasma cells in HT has been linked to higher frequency of PTC and worse clinical outcome [35]. This was also evidenced by the poorest RFS observed in PTC patients with IgG4-related HT in the present study. We found that patients with IgG4-unrelated HT experienced no recurrence whereas those with IgG4-related HT demonstrated the poorest RFS, with PTC patients having no HT exhibiting an intermediate RFS. These results offer supportive evidence on the adverse effect that IgG4-related status of HT can impose on PTC prognosis. In malignancies, IgG4 antibodies are induced to prevent immune surveillance and promote tumor cell survival [42,43]. Moreover, IgG4 has been implicated in the regulation of immune response and the suppression of inflammation. It can act as a blocking antibody, inhibiting the binding of other antibodies, including IgG1 and IgG4, to their target antigens [41,42]. This inhibitory effect may dampen the immune response in HT, possibly playing a role in sustaining chronic nature of this disease. This phenomenon is associated with unfavorable prognosis in several cancers, such as stomach cancer, melanoma, extrahepatic cholangiocarcinoma, pancreatic cancer, and esophageal cancer [42,43]. Such properties of IgG4 could offer a plausible explanation for the observed facilitation of immune escape in PTC with IgG4-related HT, potentially influencing RFS and response to therapy.

In the present study, we used digital pathology and deep learning-based image analysis software with great precision to quantify the number of IgG4-positive cells. Thus, our findings are highly reliable. However, we must acknowledge that our study has certain limitations due to its retrospective nature. In addition, it was conducted at a single institution, which might affect the generalizability of the IgG4 count threshold. Therefore, it is crucial to conduct a prospective, multicenter study to validate the universality and applicability of the IgG4 count threshold for risk stratification.

In conclusion, we identified the IgG4 count threshold as a new and clinically important marker for PTC patients with HT. This threshold can serve as a basis for future research studies that aim to validate and potentially incorporate IgG4 immunostaining into the management and prognostic assessment of thyroid cancer. With the advent of precision medicine, such biomarkers can be of great value in customizing treatment strategies for individual patients, ultimately leading to improved outcomes in PTC.

CONFLICTS OF INTEREST

No potential conflict of interest relevant to this article was reported.

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AUTHOR CONTRIBUTIONS

Conception or design: C.K.J. Acquisition, analysis, or interpretation of data: F.H., G.P., S.J., M.H., C.K.J. Drafting the work or revising: F.H., G.P., S.J., M.H., C.K.J. Final approval of the manuscript: F.H., G.P., S.J., M.H., C.K.J.

ORCID

Faridul Haq *https://orcid.org/0000-0002-0667-2786*
Gyeongsin Park *https://orcid.org/0000-0002-9727-5566*
Chan Kwon Jung *https://orcid.org/0000-0001-6843-3708*

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