Hashimoto's thyroiditis predicts outcome in intrathyroidal papillary thyroid cancer

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Abstract

Hashimoto's thyroiditis (HT) seems to have favourable prognostic impact on papillary thyroid cancer (PTC), but data were obtained analysing all disease stages. Given that HT-related microenvironment involves solely the thyroid, we aimed to assess the relationship between HT, as detected through pathological assessment, and outcome in intrathyroidal PTC. This was a multicentre, retrospective, observational study including 301 PTC with no evidence of extrathyroidal disease. Primary study endpoint was the rate of clinical remission. Auxiliary endpoint was recurrence-free survival (RFS). HT was detected in 42.5% of the cohort and was associated to female gender, smaller tumour size, lower rate of aggressive PTC variants and less frequent post-surgery radio-iodine administration. HT showed relationship with significantly higher rate of clinical remission ($P < 0.001$, OR 4, 95% CI 1.78–8.94). PTCs with concomitant HT had significantly longer RFS, as compared with non-HT tumours ($P = 0.004$). After adjustment for other parameters affecting disease outcome at univariate analysis (age at diagnosis, histology, tumour size and multifocality), prognostic effect of HT remained significant ($P = 0.006$, OR 3.28, 95% CI 1.39–7.72). To verify whether HT could optimise the identification of PTCs with unfavourable outcome, we assessed the accuracy of 'non-HT status' as negative prognostic marker, demonstrating poor capability of identifying patients not maintaining clinical remission until final follow-up (probability of no clinical remission in PTCs without HT: 21.05%, 95% CI 15.20–27.93). In conclusion, our data show that HT represents an independent prognostic parameter in intrathyroidal PTC, but cannot improve prognostic specificity.

Key Words

- ▶ thyroid carcinoma
- ▶ thyroid nodules
- ▶ Hashimoto's thyroiditis
- ▶ prognosis
- ▶ environment

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Introduction

Papillary thyroid carcinoma (PTC) is by far the most common histotype of thyroid cancer, accounting for about 80% of cases (Schlumberger 1998). Moreover, the rising incidence of thyroid malignancies, which has been recorded worldwide over the last three decades, is nearly totally attributable to increased diagnosis of the papillary histotype (Albores-Saavedra *et al.* 2007, Dal Maso *et al.* 2011, Davies & Welch 2014). This has been demonstrated for all disease stages, but tumours confined to the thyroid were those showing the higher trend (Enewold *et al.* 2009) and currently represent the most common clinical scenario for PTC. Recently, dedicated clinico-pathological systems have been developed in order to quantify the risk of persistent/recurrent disease (Pacini *et al.* 2006, Pitoia *et al.* 2013), which represents the main prognostic goal. Nevertheless, long-term risk stratification of PTC is still suboptimal, as these systems failed to demonstrate adequate positive predictive value for the identification of recurring patients (Castagna *et al.* 2011). This particularly hampers the management of intrathyroidal PTCs, in which the 10-year risk of developing structural recurrences is extremely low, ranging from 0.5 to 5% (Ito *et al.* 2012). Therefore, management of this set of PTCs is currently controversial at different steps of the therapeutic process (extent of surgery, prophylactic central node dissection and radio-iodine treatment), also due to the absence of randomised controlled trials (McLeod *et al.* 2013). Hashimoto's thyroiditis (HT) is a complex disease characterised by diffuse lymphocytic infiltration determining progressive injury of the thyroid tissue, which leads to clinically evident hypothyroidism (Antonaci *et al.* 2009). Relationship between PTC and HT has been the focus of a wide body of research aiming to find out an association between the 2 conditions and to verify if co-existing HT affects clinico-pathological features and prognosis. Although conflicting results have been produced, a huge meta-analysis performed by Lee and coworkers (Lee *et al.* 2013) found that HT was significantly more frequent in patients with PTC, as compared with subjects carrying benign lesions as well as different tumour histotypes. Furthermore, authors also pointed out that co-occurrence of HT in PTCs not only was associated to protective features such as the absence of extrathyroidal extension and lymph node metastases, but, more importantly, was related to significantly lower recurrence rates. Nevertheless, the majority of performed studies were cross-sectional and therefore unable to provide proper evaluation of clinical outcome. More importantly,

all of them included heterogeneous PTC cohorts, which were selected independently from disease extent. The aim of the present study was to define the prognostic value of histologically proven HT in intrathyroidal PTC.

Patients and methods

Patients

This was a multicentre study including 3 thyroid cancer referral units from Naples: University Federico II, University of Campania Luigi Vanvitelli and INT Pascale. In 2011, a common database was started recording clinico-pathological data of patients with differentiated thyroid cancer followed within the involved centres since 1997. Patients were registered retrospectively if operated before 2011 and prospectively thereafter. For the present study, inclusion criteria were (a) histological diagnosis of PTC (all variants were included) at pathological review; (b) intrathyroidal disease with inclusion of patients with primary tumour of any size but without extrathyroidal extension and showing no evidence of lymph nodes and distant sites involvement (subjects with post-surgery I-131 uptake outside thyroid bed were excluded) and (c) surgery and follow-up entirely performed at a single institution. Of the total 563 patients entered into the registry to date, 301 met the defined criteria. Data about clinical outcome were obtained by consulting the files and, if necessary, by recalling the patient himself. Follow-up was last updated in March 2017. The study was approved by the Ethics Committees of each included centre.

Clinical management, outcome assessment and pathological evaluation

Involved centres were tightly coordinated, with consistent treatment and follow-up procedures. The majority of intrathyroidal PTCs were treated by means of total thyroidectomy. Lymphadenectomy was not performed prophylactically, but only in case of clinical suspicious of lymph node involvement. Post-surgery RAI ablation was performed in accordance with dedicated guidelines from the referral societies (Silberstein *et al.* 2012). All patients undergoing RAI received low activity (30–50 mCi). However, RAI treatment was not advised to patients with micro-PTC (pT1a according to the TNM system), unless they harboured aggressive histology (tall cell, diffuse sclerosing and solid variants)

and/or somatic mutations with poor prognostic impact (such as the BRAFV600E). After thyroid ablation, patients were subjected to TSH suppressive therapy and underwent clinical (neck palpation), biochemical (thyroglobulin (Tg) Tg and Tg antibodies (AbTg) levels) and instrumental (neck ultrasonography (US)) follow-up every 6 months. Primary endpoint of the study was the rate of clinical remission lasting until final visit. Usually, the definition of clinical remission is consistent with the disease-free status, therefore including, as for the standard guidelines (Tuttle *et al.* 2010), patients with Tg (suppressed or stimulated) less than 1 ng/mL, negative AbTg levels, absence of suspicious finding at neck US and no pathological evidence at any other study performed for clinically indicated reasons. However, the mentioned criteria did not fit our study as a remarkable portion of patients were expected to show what is currently defined as indeterminate response to therapy (Momesso & Tuttle 2014). These include subjects undergoing surgery without RAI therapy, where Tg may show a progressive decline instead of a disappearance even when the disease is cured (Durante *et al.* 2012), and, more importantly, the study group of PTC with concomitant HT, where AbTg levels may remain positive for several years (Spencer 2011), likely due to immune memory rather than an actual disease persistence (Rubello *et al.* 1992, Dvorkin *et al.* 2013). Therefore, the definition of clinical remission in patients not treated with RAI and subjects with concomitant HT who showed detectable Tg and positive AbTg, respectively, was based on the trend of marker concentrations and the detection of clinical or instrumental evidence of disease during a sufficiently long follow-up. Based on this assumption, assessment of clinical remission was partially affected by clinicians' subjective opinion. Therefore, in order to strengthen primary endpoint analysis, we considered auxiliary endpoint recurrence-free survival (RFS). In order to make the assessment as more objective as possible and also considering that biochemical recurrence corresponds to the presence of structural disease only in 20% of cases (Haugen *et al.* 2016), definition of RFS was based on the dates of structural recurrences, defined as any new morphological evidence of disease (detected at neck US or at any other instrumental examination, such as I-131 whole-body scan, radiography, computed tomography, 18-fluorodeoxyglucose positron emission tomography or biopsy) in subjects considered as cured after thyroid ablation. Pathological diagnosis of PTC was performed according to the World Health Organization criteria (DeLellis 2004). Co-existing HT was defined by the presence of diffuse/focal lymphoplasmacytic infiltrate,

oxyphilic cells, lymphoid follicles with germinal centres and atrophic changes involving normal thyroid tissue, whereas isolated peri- and intra-tumoural lymphocytic infiltration was not considered as HT (McConahey 1972).

Statistical analysis

For all statistical analyses, SPSS, version 20.0 for Windows (SPSS Inc., Chicago, IL) was used. Group comparisons of categorical variables were performed by means of chi-square test and, in case of cell size less than 5, with Fisher's exact test (Lydersen *et al.* 2009). Cases where zeros caused problems with computation of the ORs and 95% CIs were managed by adding 0.5 to all cells of the contingency table (Pagano & Gauvreau 2000). Continuous variables, including age at diagnosis and primary tumour size, were analysed by means Mann–Whitney *U* test for medians comparison. Survival analyses were performed according to the Kaplan–Meier method, and the log-rank test was used to test for differences between groups. Multivariate models of binary logistic regression analysis were applied for assessing if co-existing HT was an independent predictor of clinical remission. Accuracy of the non-HT status as prognostic marker was assessed according to the following formula: the positive predictive value (PPV) was true positive/(true positive+false positive) and the negative predictive value (NPV) was true negative/(false negative+true negative) (Galen 1982). All tests were two sided, and *P* values of less than 0.05 were used for considering an association of statistical significance.

Results

Study cohort

Baseline clinico-pathological features and clinical outcome of the study cohort of intrathyroidal PTC were reported in Table 1. Mean follow-up was 75 ± 59 (s.d.) months (median 4.9 years). Out of 301 patients, 48 were males and 253 females. Median age at diagnosis was 38 years (range 15–71). Classic and follicular PTC was largely the most common variants accounting for 52.2 and 25.9% of cases, respectively. Nevertheless, the portion of aggressive/unfavourable variants was not negligible, including 28 cases (13 tall cell, 10 diffuse sclerosing and 5 solid PTCs) (9.3%). The latter were the only patients to be considered at intermediate risk of recurrence/persistence according to the America Thyroid Association criteria (Pitoia *et al.* 2013), whereas the vast majority of the cohort (90.7%) was composed of

Table 1 Baseline clinico-pathological characteristics and prognostic outcome of the overall cohort of intrathyroidal PTC.

Parameter	Value
Number of patients	301
Age at diagnosis, median (range)	38 (15–71)
Sex-ratio, males/females, number (%)	48/253 (15.9/84.1)
PTC variants, number (%)	
Classic	157 (52.2)
Follicular	78 (25.9)
Warthin-like	24 (8.0)
Hürthle cells	14 (4.7)
Tall cell	13 (4.3)
Diffuse sclerosing	10 (3.3)
Solid	5 (1.7)
Primary tumour size, median (range) (cm)	1.2 (0.6–4)
Micro-PTC, number (%)	117 (38.9)
Multifocality, number (%)	76 (25.2)
Co-existing HT, number (%)	128 (42.5)
No post-surgery RAI-treatment, number (%)	68 (22.6)
ATA risk group, low/intermediate/high, number (%)	273/28/0 (90.7/9.3/0.0)
Clinical remission, number (%)	
Yes	255 (84.7)
Disease-free	236 (78.4)
Detectable Tg*	0 (0.0)
AbTg positivity**	19 (6.3)
No	44 (13.3)
Structural recurrence	32 (10.6)
Rising Tg	12 (4.0)
Rising AbTg	0 (0.0)
Uncertain***	2 (0.7)

*Patients not subjected to post-surgery RAI showing stable/declining Tg levels with no other evidence of disease; **Patients with concomitant HT showing stable/declining AbTg levels with no other evidence of disease; ***Patients with non-specific imaging findings.

ATA, American Thyroid Association; HT, Hashimoto's thyroiditis; PTC, Papillary thyroid cancer; RAI, Radio-iodine.

Table 2 Comparison of clinico-pathological features between patients without and with co-existing HT in the study cohort of intrathyroidal PTC.

Clinico-pathological features	PTC without co-existing HT	PTC with co-existing HT	OR (95% CI)*	P value
Age at diagnosis, median (range)	37 (15–71)	39.5 (17–64)	na	0.195
Gender, females, number (%)	133/173 (76.9)	120/128 (93.8)	4.51 (2.03–10.02)	<0.001
PTC variants, number (%)				
Classic	96/173 (55.5)	61/128 (47.7)		<0.001
Follicular	36/173 (20.8)	42/128 (32.8)		
Hürthle cells	9/173 (5.2)	5/128 (3.9)		
Warthin-like	6/173 (3.5)	18/128 (14.1)		
Tall cell	12/173 (6.9)	1/128 (0.8)		
Solid	9/173 (5.2)	1/128 (0.8)		
Diffuse sclerosing	5/173 (2.9)	0/128 (0)		
Primary tumour size, median (range) (cm)	1.3 (0.7–4)	1 (0.6–4)	na	0.008
Multifocality, number (%)	50/173 (28.9)	26/128 (20.3)	0.64 (0.37–1.1)	0.105
Post-surgery RAI-treatment, number (%)	143/173 (82.7)	82/128 (64.1)	0.40 (0.23–0.69)	0.001

*ORs with 95% CI were calculated using PTC without co-existing HT as reference.

CI, Confidence interval; HT, Hashimoto's thyroiditis; OR, Odds ratio; PTC, Papillary thyroid cancer; RAI, Radio-iodine.

low-risk PTCs. Histologically proven HT was detected in 128 subjects (42.5%). Among them, 118 (92.2%) were AbTg positive before surgery, and the vast majority (99, 83.9%) achieved negativisation during follow-up with a median disappearing time of 17 ± 4.1 months. Sixty-eight patients (22.6%), all affected with micro-PTC, were not advised to RAI. Overall, 255 (84.7%) patients were classified as being in clinical remission until final visit. Of them, the vast majority (236, 78.4%) met full criteria for the disease-free status, whereas the remaining portion (19, 16.1%) included patients with concomitant HT who showed stable/declining AbTg levels and no other evidence of disease after a mean follow-up of 63 ± 42 (s.d.) months (median 4.1 years). Among patients classified not in clinical remission, 32 (10.6%) experienced documented structural recurrence, whereas 12 (4%) showed rising Tg levels.

Relationship between HT and clinico-pathological features at diagnosis

Correlation between pathologically proven HT and baseline characteristics were reported in Table 2. A wide predominance of the female gender was observed in patients with HT, as compared with the control group ($P < 0.001$, OR 4.51, 95% CI 2.03–10.02). Primary tumour size was significantly smaller in case of concomitant HT (median 1 vs 1.3 cm; $P = 0.008$). A strong correlation was found between HT and PTC histology ($P < 0.001$). In order to characterise such relationship, we performed a multiple testing analysis assessing the association between HT and different PTC histotypes (Table 3). We found that patients with HT were more likely affected

Table 3 Comparison of single PTC histotypes between patients without and with co-existing HT in the study cohort of intrathyroidal PTC.

PTC variants	PTC without co-existing HT	PTC with co-existing HT	OR (95% CI)	Unadjusted <i>P</i> value	Adjusted <i>P</i> value
Classic	96/173 (55.5)	61/128 (47.7)	0.73 (0.46–1.15)	0.178	0.356
Follicular	36/173 (20.8)	42/128 (32.8)	1.85 (1.1–3.12)	0.018	0.054
Hürthle cells	9/173 (5.2)	5/128 (3.9)	0.74 (0.24–2.26)	0.596	0.596
Warthin-like	6/173 (3.5)	18/128 (14.1)	4.58 (1.76–11.9)	<0.001	<0.001
Aggressive histology*	26/173 (15)	2/128 (1.6)	0.08 (0.02–0.38)	<0.001	<0.001

*Owing to the low number of cases, the aggressive tall cell, diffuse sclerosing, and solid PTC variants were considered as a single group. *P* values have been adjusted by means of the Holm-Bonferroni correction.

HT, Hashimoto's thyroiditis; PTC, Papillary thyroid cancer.

with the favourable Warthin-like ($P<0.001$, OR 4.58, 95% CI 1.76–11.9) and follicular ($P=0.018$, OR 1.85, 95% CI 1.1–3.12) variants, whereas the risk of aggressive histology was significantly lower ($P<0.001$, OR 0.08, 95% CI 0.02–0.38), as compared with the control group. Nevertheless, significance of the association with the follicular variant was lost after correction by means of the Holm-Bonferroni method (Holm 1979). PTC with co-existing HT were less frequently subjected to post-surgery administration of I-131 ($P=0.001$, OR 0.4, 95% CI 0.23–0.69), due to the reduced tumour volume.

Relationship of HT with clinical remission and RFS

Two patients with non-specific imaging findings were excluded from outcome analysis. Concomitant HT was

associated to significantly higher likelihood of being in clinical remission during the whole follow-up time ($P<0.001$, OR 4, 95% CI 1.78–8.94), and this represented our primary endpoint. Analysis of RFS, considered as auxiliary endpoint, included 295 subjects, as the time of recurrence was not unequivocally defined for 4 subjects. As reported in Fig. 1, PTCs with concomitant HT had significantly longer RFS, as compared with non-HT tumours ($P=0.004$).

Multivariate analysis of prognostic factors of clinical remission

In order to test whether concomitant HT was an independent predictor of clinical remission, we built a multivariate adjusted model basing on clinico-pathological features with possible prognostic impact on intrathyroidal PTC. Results were reported in Table 4. At univariate analysis, parameters other than HT showing relationship with clinical remission were age at diagnosis ≥ 45 years ($P<0.001$, OR 9.42, 95% CI 2.22–39.91), unfavourable PTC histology ($P=0.001$, OR 0.25, 95% CI 0.11–0.6), microcarcinoma ($P<0.001$, OR 4.88, 95% CI 1.99–11.95), multifocality ($P<0.001$, OR 0.27, 95% CI 0.14–0.52) and post-surgery RAI ($P<0.001$, OR 0.02, 95% CI 0–0.48). Notably, RAI-treatment exerted a paradoxical deleterious prognostic impact being associated to lower likelihood of clinical remission. This result cannot be considered as demonstrating an actual prognostic effect for post-surgery RAI, but was rather due the fact that all patients not subjected to RAI were affected with micro-PTC and had therefore less aggressive disease. Owing to this consideration, post-surgery RAI was excluded from our multivariate model. Multivariate analysis confirmed co-existing HT as predictor of clinical remission ($P=0.006$, OR 3.28, 95% CI 1.39–7.72), together with age at diagnosis ≥ 45 years ($P=0.023$, OR 5.57, 95% CI 1.26–24.53) and microcarcinoma ($P=0.010$, OR 3.40, 95% CI 1.33–8.66).

Figure 1 Kaplan-Meier analysis of recurrence-free survival according to co-existing HT. HT, Hashimoto's thyroiditis.

Table 4 Multivariate analysis of prognostic factors of clinical remission in the cohort of intrathyroidal PTC.

	Clinical remission, N (%)				Adjusted multivariate model	
	Yes	No	Unadjusted OR (95% CI)	Unadjusted P value	Adjusted OR (95% CI)***	Adjusted P value
Gender						
Male	38 (79.2)	10 (20.8)	1 (reference)	0.192	Not included	Not included
Female	217 (86.5)	34 (13.5)	1.67 (0.76–3.68)			
Age at diagnosis						
<45 years	176 (80.7)	42 (19.3)	1 (reference)	<0.001	1 (reference)	0.023
≥45 years	79 (97.5)	2 (2.5)	9.42 (2.22–39.91)		5.57 (1.26–24.53)	
Histology*						
Favourable	237 (87.5)	34 (12.5)	1 (reference)	0.001	1 (reference)	0.483
Unfavourable	18 (64.3)	10 (35.7)	0.25 (0.11–0.60)		0.69 (0.25–1.91)	
Tumour size**						
Microcarcinoma	111 (94.9)	6 (5.1)	4.88 (1.99–11.95)	<0.001	3.40 (1.33–8.66)	0.010
Macrocarcinoma	144 (79.1)	38 (20.9)	1 (reference)		1 (reference)	
Multifocality						
Yes	54 (71.1)	22 (28.9)	0.27 (0.14–0.52)	<0.001	0.47 (0.21–1.02)	0.056
No	119 (90)	22 (10)	1 (reference)		1 (reference)	
Co-existing HT						
Yes	120 (93.8)	8 (6.2)	4.0 (1.78–8.94)	<0.001	3.28 (1.39–7.72)	0.006
No	135 (78.9)	36 (21.1)	1 (reference)		1 (reference)	
Post-surgery RAI						
Yes	179 (80.3)	44 (19.7)	0.02 (0–0.48)	<0.001	Not included	Not included
No	68 (100)	0 (0)	1 (reference)			

*Favourable PTC histology includes classic, follicular, Warthin-like, and Hurtle-cells variants; unfavourable histology includes tall cell, diffuse sclerosing, and solid variants; **Categorisation in micro- and macro- carcinoma was used for the analysis.
CI, Confidence interval; HT, Hashimoto's thyroiditis; N, number; OR, Odds ratio; RAI, Radio-iodine.

Utility of the parameter HT in predicting poor clinical outcome

Since the aim of PTC prognostication was to identify the subgroup of patients with unfavourable outcome, whereas HT revealed positive prognostic impact, we analysed the performance of the non-HT status as prognostic marker, specifically assessing its capability in identifying those subjects not maintaining clinical remission until the final visit. Despite limited by the low prevalence of the disease status, namely the absence of clinical remission, which was 14.7%, the absence of HT showed limited specificity, namely 47.06% (95% CI 40.80–53.38), and poor PPV, namely 21.05% (95% CI 15.20–27.93). This suggests very limited utility of the parameter HT for assessing the risk of persistent/recurrent disease.

Discussion

The development of an immune response has been reported in a significant portion of PTC, as demonstrated by the presence of inflammatory infiltration within or surrounding the tumour (French *et al.* 2010). Whether this immune microenvironment has an impact on PTC

development and progression, and the underlying molecular mechanisms, is still a matter of discussion. Indeed, PTC-related immune network is extremely complex involving a myriad of cells (different lymphocyte subtypes, macrophages, mast cells and dendritic cells) and also a variety of soluble factors (cytokines, chemokines, angiogenic and lymphangiogenic factors) (Galdiero *et al.* 2016). To date, available data pointed out a relationship between intra- and/or peri- tumoural inflammatory activation, mainly assessed by the presence of infiltrating lymphocytes, and favourable outcome, namely a reduced risk of disease recurrence (Matsubayashi *et al.* 1995, Gupta *et al.* 2001). Owing to these considerations, possible impact of co-existing HT on PTC outcome is an intriguing issue. Indeed, HT relies on an aberrant immune reaction specifically directed against the thyroid, which involves several immune cells as well as soluble mediators and leads to wide inflammatory infiltration of the gland (Weetman 2013). Actually, it is still controversial whether the previously mentioned tumour infiltrating lymphocytes represents an independent entity having own prognostic significance, as reported by French and coworkers (French *et al.* 2010), or has to be considered as inserted in the context of concomitant HT, as suggested by Kebebew and coworkers (Kebebew *et al.* 2001). Among a series of

prognostic studies having sufficiently long follow-up, the vast majority reported more favourable outcome for PTC when co-existing HT was detected (Matsubayashi *et al.* 1995, Loh *et al.* 1999, Kashima *et al.* 1998, Kebebew *et al.* 2001, Kim *et al.* 2009, Ahn *et al.* 2011, Huang *et al.* 2011, Jeong *et al.* 2012, Dvorkin *et al.* 2013). Nevertheless, HT revealed as an independent prognostic factor only in some of them (Kashima *et al.* 1998, Loh *et al.* 1999, Dvorkin *et al.* 2013). However, prognostic value of HT in PTC is still considered controversial because of the following issues: (a) some of the mentioned papers involved mainly but not exclusively the papillary histotype (Loh *et al.* 1999, Dvorkin *et al.* 2013); (b) criteria for HT definition were heterogeneous: some authors included not only patients with full histopathological features of HT (as previously described McConahey 1972), but also subjects with local tumour lymphocytic infiltration alone (Loh *et al.* 1999, Kim *et al.* 2009), whereas some others considered both clinics and histology for HT diagnosis (Dvorkin *et al.* 2013); (c) all studies were focused on heterogeneous cohorts involving all grades of disease extent. In our opinion, the latter was a crucial limitation as the interaction between HT-related microenvironment and PTC cells occurs solely within the thyroid, so the actual effect of HT on tumour evolution could be lost or at least diluted when the disease goes behind the gland. Furthermore, it is conceivable that PTC with HT is diagnosed at an earlier stage because of the more accurate ultrasonographic screening, and this may hamper the proper evaluation of the correlation between HT and tumour behaviour. To the best of our knowledge, this is the first study reporting significant data about the prognostic impact of concomitant HT on a large PTC cohort with intrathyroidal disease. Previously, Matsubayashi and coworkers (Matsubayashi *et al.* 1995) have tried to determine the prognostic role of local tumour lymphocytic infiltration according to the extent of PTC at the time of initial treatment, but sample size was too low for obtaining significant results. Another crucial characteristic of our study was that HT definition was based solely on histology. Particularly, only patients showing the typical histopathological changes in the area of normal thyroid parenchyma were included in the co-existing HT group, whereas those with localised tumour lymphocytic infiltration alone were considered as controls. In the present study, co-existing HT was detected in 42.5% of PTC, a prevalence higher than that reported in any other series (ranging from 0.5 to 38%) (Lee *et al.* 2013). This could be explained by the association between HT and less advanced disease stage, which has been

reported in many studies (Loh *et al.* 1999, French *et al.* 2010, Huang *et al.* 2011). Another possible reason is that the majority of patients lived in a highly polluted area such as Naples and the neighbour countries (Grumetto *et al.* 2015), and overexposure to thyroid-disrupting chemicals may facilitate the development of the autoimmune process in subjects predisposed to HT (Colucci *et al.* 2015). Analysis of baseline features revealed that primary tumour size was smaller in case of concomitant HT. This is an interesting finding as the majority of previous studies including all PTC stages failed to demonstrate this association (Lee *et al.* 2013). It may be hypothesised that analysing only intrathyroidal disease actually reveals the counteracting action of HT against local tumour growth. Even more interestingly, we also demonstrated a relationship between HT and PTC histology. Particularly, patients with concomitant HT showed significantly lower risk of aggressive variants. Furthermore, a strong relationship was found with the favourable Warthin-like and, even if statistical significance was lost after correction with the Holm–Bonferroni method, follicular PTC. However, assessment of relationship with the follicular variant was also hampered by the fact that histological definition pre-dated the recent reclassification of encapsulated tumours such as a ‘noncancerous or premalignant neoplasm’, named ‘noninvasive follicular thyroid tumour with papillary-like nuclear features’ (NIFTP). With the exception of the strong association with the Warthin-like variant, which has been long established (Baloch & LiVolsi 2000), no evidence about the relationship between HT and PTC histology had been reported before. Analysis of clinical outcome revealed that concomitant HT was associated to higher likelihood of achieving and maintaining clinical remission until final visit. Furthermore, PTCs with HT showed higher RFS at survival analysis. Prognostic value of concomitant HT was further assessed by adjusting for parameters showing significant relationship with clinical remission at univariate analysis. These include age at diagnosis, primary tumour size, multifocality, histology and post-surgery RAI. Although deleterious effect related to higher tumour size and the presence of multifocality as well as aggressive histology was expected and already reported (Ito *et al.* 2012, Ghossein *et al.* 2014), protective role of older age (≥ 45 years) and deleterious effect of post-surgery RAI appeared controversial. Actually, the former was consistent with the recent Ito study (Ito *et al.* 2014) showing that patients with non-operated low-risk PTC have lower risk of disease progression when age is ≥ 40 years, whereas the latter can be interpreted as

exclusively related to the strict correlation with tumour size and was therefore excluded from the multivariate model. After adjustment, co-existing HT retained its protective effect, and this represents the main finding of our study. Recently, the Thyroid Cancer Genome Atlas has identified two main PTC groups with different genetics, pathology and clinical behaviour, namely RAS- and BRAF-mutated tumours (Agrawal et al. 2014). Considering our findings, it may be speculated that HT-related PTC represents an independent clinical entity belonging to the RAS-mutated group, which predominantly includes the follicular variant histology and has better outcome, whereas the BRAF-mutated tumours involve the vast majority of aggressive variants and show more aggressive behaviour. However, this remains just a speculation and further studies, also including molecular characterisation, are needed to confirm this thesis. To date, HT is not included within the subset of variables to be considered for estimating clinical outcome of PTC (Haugen et al. 2016). Results from this study not only empower the thesis of a prognostic value of HT in PTC, but also provide insights about the possible application into clinical practice. Indeed, based on the current prognostic criteria (Pacini et al. 2006, Pitoia et al. 2013), our study population of intrathyroidal PTC was mainly composed of tumours with low-risk of recurrent/persistent disease, with the only exception of those few patients showing aggressive histology, who had to be collocated in the intermediate-risk class. Therefore, our findings can be considered as substantially referring to the setting of low-risk PTC, whereas further studies are needed to assess prognostic value of HT in other risk categories. Since the capability of identifying PTC with less favourable outcome represents the main limitation of current PTC prognostication, especially in patients with early disease, we analysed the accuracy of the non-HT status, considered as a negative prognosticator. We found that the absence of HT had low specificity and PPV for the identification of subjects not showing remission until the final visit, thus demonstrating the limited utility of the HT parameter for the actual improvement of PTC prognostication. In conclusion, we demonstrated that concomitant HT, as detected through pathological examination, is an independent prognostic factor in intrathyroidal PTC, but cannot improve prognostic specificity.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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