

Hashimoto's thyroiditis is associated with papillary thyroid carcinoma: role of TSH and of treatment with L-thyroxine

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Abstract

The possible association between Hashimoto's thyroiditis (HT) and papillary thyroid carcinoma (PTC) is a still debated issue. We analyzed the frequency of PTC, TSH levels and thyroid autoantibodies (TAb) in 13 738 patients (9824 untreated and 3914 under L-thyroxine, L-T₄). Patients with nodular-HT ($n=1593$) had high titer of TAb and/or hypothyroidism. Patients with nodular goiter (NG) were subdivided in TAb – NG ($n=8812$) with undetectable TAb and TAb + NG ($n=3395$) with positive TAb. Among untreated patients, those with nodular-HT showed higher frequency of PTC (9.4%) compared with both TAb – NG (6.4%; $P=0.002$) and TAb + NG (6.5%; $P=0.009$) and presented also higher serum TSH (median 1.30 vs 0.71 $\mu\text{U/ml}$, $P<0.001$ and 0.70 $\mu\text{U/ml}$, $P<0.001$ respectively). Independently of clinical diagnosis, patients with high titer of TAb showed a higher frequency of PTC (9.3%) compared to patients with low titer (6.8%, $P<0.001$) or negative TAb (6.3%, $P<0.001$) and presented also higher serum TSH (median 1.16 vs 0.75 $\mu\text{U/ml}$, $P<0.001$ and 0.72 $\mu\text{U/ml}$, $P<0.001$ respectively). PTC frequency was strongly related with serum TSH (odds ratio (OR)=1.111), slightly related with anti-thyroglobulin antibodies (OR=1.001), and unrelated with anti-thyroperoxidase antibodies. In the L-T₄-treated group, when only patients with serum TSH levels below the median value (0.90 $\mu\text{U/ml}$) were considered, no significant difference in PTC frequency was found between nodular-HT, TAb – NG and TAb + NG. In conclusion, the frequency of PTC is significantly higher in nodular-HT than in NG and is associated with increased levels of serum TSH. Treatment with L-T₄ reduces TSH levels and decreases the occurrence of clinically detectable PTC.

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Introduction

Hashimoto's thyroiditis (HT) is frequently diagnosed especially in females and is the most common cause of hypothyroidism in iodine-sufficient areas of the world, with an increasing prevalence in older patients (Hollowell *et al.* 2002). An association has been suggested between HT and papillary thyroid carcinoma (PTC) in many studies (Dailey *et al.* 1955, Hirabayashi & Lindsay 1965, Baker 1995, Okayasu 1997, Singh *et al.* 1999), even if other studies yielded conflicting results (Holm *et al.* 1985, Anil *et al.* 2010). The link between HT and PTC is supported by the observation that rearrangements of RET oncogene (RET/PTC),

frequently detected in PTC, may also be found in the thyroids of patients affected by HT, with no histopathological evidence of PTC (Mechler *et al.* 2001), but these data have been criticized for technical limitations of the methods used (Nikiforov 2006, Rhoden *et al.* 2006).

The heterogeneity of criteria used to define HT may hamper the drawing of conclusions on this issue. By strict criteria, HT is a histological diagnosis characterized by widespread lymphocytic infiltration of the thyroid. On the other hand, PTC is often associated with a significant lymphocytic infiltration in the absence of the typical signs of autoimmune thyroiditis (Okayasu *et al.* 1995, Fiore *et al.* 2009b), and may

represent a response to tumor antigens released through disruption of normal follicles by neoplastic invasion. On clinical grounds, the diagnosis of HT is based on the presence of serum thyroid autoantibodies (TAB) and of spontaneous hypothyroidism that may be present at the initial evaluation or may develop during follow-up (Vanderpump *et al.* 1995, Walsh *et al.* 2010). However, patients with nodular goiter (NG) may also have circulating TAB as expression of focal thyroiditis not evolving toward hypothyroidism, thus making the diagnosis of HT cumbersome.

The progressive reduction of thyroid function as a consequence of the autoimmune process leads to a progressive increase in serum TSH. Recently, it has been reported that in patients with nodular thyroid diseases, the risk of thyroid malignancy increases with serum TSH concentrations (Boelaert *et al.* 2006, Haymart *et al.* 2008a,b, Jonklaas *et al.* 2008, Polyzos *et al.* 2008, Fiore *et al.* 2009a, Jin *et al.* 2010). Therefore, it is possible to hypothesize that increased TSH levels may play a role in the development of PTC also in patients with nodular-HT.

The results reported in this paper show that PTC is more frequent in patients with nodular-HT compared with patients with non-autoimmune NG and that the increased TSH levels, a consequence of the destruction of functioning tissue by the autoimmune process, are strictly related to the increased frequency of PTC. In agreement with this conclusion, treatment with L-thyroxine (L-T₄) reduces TSH levels and decreases the frequency of PTC in nodular-HT.

Methods and patients

Methods

Free thyroid hormones (FT₄ and FT₃) were measured by a chemiluminescent assay (Vitros Ortho-Clinical Diagnostics Johnson and Johnson Company, High Wycombe, UK normal range FT₄ 7–17 pg/ml and FT₃ 2.7–5.7 pg/ml). TSH was measured by a chemiluminescent assay (Immulite 2000 Siemens, Llanberis, UK – normal range 0.4–3.4 μU/ml). Thyroglobulin antibodies (TgAb) and thyroperoxidase antibodies (TPOAb) were measured by an immunoenzymatic assay (AIA-Pack TgAb, and TPOAb, Tosoh, Tokyo, Japan) and considered positive when >30 U/ml for TgAb and >10 U/ml for TPOAb.

Ultrasound evaluation of the thyroid gland was carried out using a commercially available real-time instrument (Technos, Esaote Biomedica, Genova, Italy) using a 7.5–10 MHz linear transducer. The echodensity of the thyroid was also examined and

defined in comparison with the anatomic structures that are isoechoic (submandibular glands) or hypoechoic (neck muscles) with respect to the normal thyroid tissue. Thyroid scintiscan was performed in all patients with multinodular goiter and in patients with single nodules when they had low serum TSH (<0.4 μU/ml). Fine needle aspiration (FNA) was performed in all nodules 'cold' at scintiscan larger than 1 cm or in smaller nodules if suspicious features were detected at thyroid ultrasound.

Patients

In this study, we included 13 738 patients at their first observation in our Department between 2004 and 2009 who underwent FNA of cold thyroid nodules and who fulfilled the following criteria:

- they had TSH, free thyroid hormones and serum TgAb and TPOAb measured simultaneously with FNA;
- they had a diagnostic cytological exam (patients with non-diagnostic or indeterminate cytology were excluded);
- Graves' disease was excluded according to the standard diagnostic criteria (hyperthyroidism with or without ophthalmopathy, positive serum TSH-receptor antibodies and/or TgAb and TPOAb).

Out of the 13 738 patients, 9824 were untreated and 3914 under treatment with L-T₄ for NG or for hypothyroidism (L-T₄-treated patients).

Patients were diagnosed as affected by nodular-HT when:

- they had high titer of TAB (>100 U/ml of both TgAb and TPOAb) or were hypothyroid;
- they had positive TAB not fulfilling the criteria reported in point a), but presented a clear hypoechoic 'thyroiditis' pattern at thyroid ultrasound.

In the untreated group a clinical diagnosis of nodular-HT was performed 893/9824 (9.1%) patients: 760 (85.1%) according to the criteria reported in point a) and 133 (14.9%) according to the criteria of point b). In the L-T₄-treated group nodular-HT was diagnosed in 638/3914 (16.3%) patients. Out of these 638 patients, 388 (60.8%) were treated with L-T₄ for hypothyroidism and 250 (39.2%), who had high titer of TAB, although being euthyroid, were treated with L-T₄ for nodular thyroid disease.

Patients with NG had single or multiple nodules and did not present a ‘hypoechoic’ pattern at thyroid ultrasound. They were euthyroid or presented sub-clinical hyperthyroidism and had cold nodules or both cold and ‘hot’ nodules at thyroid scan. NG patients either had undetectable serum TAb (TAb–NG group, $n=8812$) or had positive serum TAb, but did not satisfy the criteria used for the definition of nodular-HT (TAb+NG group $n=3395$). Out of the 9824 untreated patients, 6571 (66.9%) were included in the TAb–NG group and 2360 (24.0%) in the TAb+NG group. Out of the 3914 L-T₄-treated patients, 2241 (57.3%) were included in the TAb–NG group and 1035 (26.4%) in the TAb+NG group.

FNA, cytological and histological diagnosis

FNA was performed under echo guidance using a 23-gauge needle attached to a 10 ml syringe. The material was air-dried, stained with Papanicolaou and Giemsa and interpreted by an experienced cytologist (G D C). The adequacy of aspirates was defined according to the guidelines of the Papanicolaou Society (The Papanicolaou Society of Cytopathology Task Force on Standards of Practice 1996) and cytological results were classified according to the criteria of the British Thyroid Association (2007).

Out of the 13 738 patients, 12 890 had one or more nodules with a benign cytology and 848 at least one nodule with a cytology suggestive or indicative of PTC. Out of the 848 patients with a cytological diagnosis of PTC, 677 were submitted to thyroidectomy in our Department. For histological diagnosis, formalin-fixed, paraffin-embedded nodular tissues

were stained by hematoxylin and eosin and the diagnosis was made according to the World Health Organization guidelines. The diagnosis of PTC was confirmed by histology in 666/677 patients (98.3%); 3/677 (0.4%) patients had a histological diagnosis of medullary thyroid cancer, 2/677 (0.2%) metastasis of lung cancer and 6/677 (0.9%) follicular adenoma.

Statistical analysis

TSH values were expressed as median and interquartile range (IR). Non-parametric tests (χ^2 , or Mann–Whitney U test) were used as appropriate and considered statistically significant when $P < 0.05$. For the multivariate analysis, a forward stepwise binary logistic regression was performed to identify the significant predictors of PTC. The Nagelkerke R^2 was used to assess how well the model fitted.

Results

Clinical and histological features of patients with PTC

The histological and clinical features of patients with PTC are summarized in Table 1. The frequency of female patients was significantly lower in TAb–NG group than in TAb+NG ($P < 0.0001$) and in nodular-HT ($P = 0.01$) groups. Nodular-HT patients were significantly younger than TAb–NG ($P < 0.003$) and TAb+NG ($P = 0.02$) patients. Furthermore, the frequency of node metastasis was significantly higher in nodular-HT than in TAb–NG ($P = 0.03$), but was not significantly different between nodular-HT and TAb+NG patients. In TAb+NG the frequency of node

Table 1 Clinical and histological features of papillary thyroid carcinoma patients

	TAb–NG ($n=399$)	TAb+NG ($n=155$)	Nodular-HT ($n=112$)	All ($n=666$)
% F ^a	68.4	85.0	81.3	73.3
Age (years) ^b	44.2 ± 15.1	43.3 ± 13.4	39.6 ± 13.1	43.2 ± 14.5
Histological variant (%)				
Classic	57.7	52.7	53.2	55.8
Tall cell	12.1	16.0	16.5	13.8
Follicular	14.7	14.0	14.7	14.5
Mixed form	14.7	12.7	14.7	14.2
Other	0.8	4.7	0.9	1.7
T (%)				
T1	36.2	42.3	41.3	38.5
T2	13.1	12.8	11.9	12.8
T3–4	50.6	44.7	46.8	48.7
Node pos (%) ^c	25.3	33.1	35.5	28.8

^aFrequency of female patients significantly lower in TAb–NG than in TAb+NG ($P < 0.0001$) and in nodular-HT ($P = 0.01$).

^bNodular-HT patients significantly younger than TAb–NG ($P < 0.003$) and TAb+NG ($P = 0.02$) patients.

^cFrequency of metastatic nodes significantly higher in nodular-HT than in TAb–NG ($P = 0.03$).

metastasis was higher, but not statistically different compared with TAB–NG. No significant difference was observed in frequency of the different histological variants of PTC and the size of primary tumor in relation with the clinical diagnosis.

Frequency of PTC according to serum TSH and clinical diagnosis in untreated patients

In nodular-HT patients, the frequency of PTC (84/893, 9.4%) was significantly higher compared with both TAB–NG (421/6571, 6.4%; $\chi^2 P=0.008$) and TAB+NG (154/2360, 6.5%; $\chi^2 P=0.005$) patients, while no difference was observed between TAB–NG and TAB+NG patients (Fig. 1, panel A). Nodular-HT patients also showed higher serum TSH (1.30 $\mu\text{U/ml}$, IR 0.61–2.37 $\mu\text{U/ml}$) compared with both TAB–NG (0.71 $\mu\text{U/ml}$, IR 0.40–1.10 $\mu\text{U/ml}$, Mann–Whitney U test $P\leq 0.001$) and TAB+NG (0.70 $\mu\text{U/ml}$, IR

0.37–1.20 $\mu\text{U/ml}$, Mann–Whitney U test $P<0.001$) patients (Fig. 1, panel B).

The clinical diagnosis of nodular-HT in this series of patients was based on the presence of hypothyroidism and/or high TAb levels. In Table 2, all patients with high TAb ($n=681$) were included in the nodular-HT group. Among patients with low titer of serum TAb ($n=2572$), 212 had a clinical diagnosis of nodular-HT (79 were hypothyroid and 133, although being euthyroid, presented a clearly hypoechoic gland at thyroid ultrasound) and 2360 were included in the TAB+NG group (because they were euthyroid with no hypoechoic pattern at thyroid ultrasound). All patients with negative TAb ($n=6571$) were included in the TAB–NG group. Independently from the clinical diagnosis the frequency of PTC was significantly higher in patients with high titer of serum TAb (63/681, 9.3%) with respect with those with low titer of serum TAb (175/2572, 6.8%; $\chi^2 P<0.001$) and negative TAb (421/6571, 6.3%; $\chi^2 P<0.001$). Patients with high serum TAb also showed higher serum TSH (1.16 $\mu\text{U/ml}$, IR 0.52–2.00 $\mu\text{U/ml}$) compared with both patients with low TAb (0.75 $\mu\text{U/ml}$, IR 0.39–1.34 $\mu\text{U/ml}$, Mann–Whitney U test $P<0.001$) and patients with negative TAb (0.72 $\mu\text{U/ml}$, IR 0.40–1.10 $\mu\text{U/ml}$, Mann–Whitney U test $P\leq 0.001$) patients.

A forward stepwise binary logistic regression was performed to identify the variables independently associated with an increased frequency of PTC. TSH levels, serum levels of TgAb and TPOAb were considered in the logistic regression model. The probability of PTC significantly increases with increasing serum TSH levels (odds ratio (OR)=1.111 per 1 $\mu\text{U/ml}$, 95% confidence interval (CI): 1.048–1.177). TgAb levels conferred a further small, but significant increase in risk of PTC (OR=1.001 per U/ml, CI: 1.000–1.002), while TPOAb were completely unrelated to PTC. The logistic model was significant, with a P value lower than 0.01 (Nagelkerke $R^2=0.8\%$) after the inclusion of the predictors.

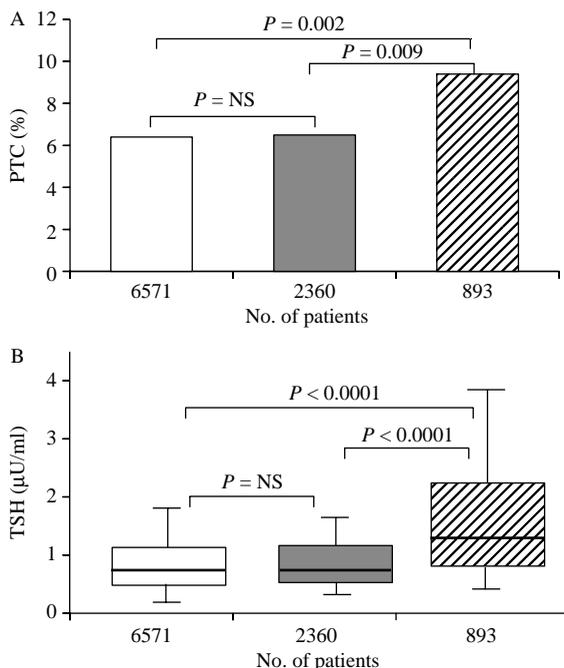


Figure 1 (Panel A) Frequency of PTC in patients with a clinical diagnosis of TAB–NG (white column), TAB+NG (gray column), and nodular-HT (striped column). Nodular-HT patients showed a significantly higher frequency of PTC compared with both TAB–NG and TAB+NG, while no difference was observed between TAB–NG and TAB+NG patients ($\chi^2 P$ value reported in figure). (Panel B) Box–whisker plots of serum TSH ($\mu\text{U/ml}$) in patients with TAB–NG (white column), TAB+NG (gray column), and nodular-HT (striped column). Results are reported as median values (black lines), interquartile (25th–75th percentiles) range (boxes) and 10th–90th percentiles (whiskers). The statistical difference between groups was evaluated by the Mann–Whitney U test. Nodular-HT patients showed significantly higher TSH levels compared with both TAB–NG and TAB+NG patients (P value reported in figure).

Frequency of PTC according to serum TSH and clinical diagnosis in L-T₄-treated patients

In L-T₄-treated group, nodular-HT patients showed higher serum TSH (0.81 $\mu\text{U/ml}$, IR 0.30–2.87 $\mu\text{U/ml}$) compared with both TAB–NG (0.33 $\mu\text{U/ml}$, IR 0.13–0.65 $\mu\text{U/ml}$, Mann–Whitney U test $P\leq 0.001$) and TAB+NG (0.40 $\mu\text{U/ml}$, IR 0.15–0.83 $\mu\text{U/ml}$, Mann–Whitney U test $P<0.001$) patients (Fig. 2, panel A). This result may be explained by the different purpose of the L-T₄ treatment. Indeed, in all NG patients the objective of L-T₄ therapy was to reduce TSH levels

Table 2 Frequency of papillary thyroid carcinoma (PTC) according to serum TSH and thyroid autoantibodies (TAb) in untreated patients

TAb titer	Clinical diagnosis		Serum TSH (μ U/ml) (median and IR)	PTC
	Nodular-HT	NG		
High TAb	681	0	1.16 (0.52–2.00) ^a	63/681 (9.3%) ^d
Low TAb	212	2360	0.75 (0.39–1.34) ^b	175/2572 (6.8%) ^e
Negative TAb	0	6571	0.72 (0.40–1.10) ^c	421/6633 (6.3%) ^f

Nodular-HT, nodular Hashimoto's thyroiditis; NG, nodular goiter; Negative TAb, undetectable serum TgAb and TPOAb; High TAb, high titer (> 100 U/ml) of both TgAb and TPOAb; Low TAb, positive TgAb and/or TPOAb not fulfilling the criteria indicated in High TAb. a versus b and versus c, Mann–Whitney U test $P \leq 0.001$; b versus c, Mann–Whitney U test P NS. d versus e and versus f: χ^2 $P < 0.001$; e versus f: χ^2 P NS.

to the lower values of normal range, while in 388/638 (60.8%) nodular-HT patients the purpose was to correct hypothyroidism bringing serum TSH into the range of normal values.

The frequency of PTC was significantly higher in nodular-HT patients (55/638, 8.6%) compared with both TAb–NG (82/2241, 3.7%; χ^2 $P < 0.001$) and TAb+NG (52/1035, 5.0%; χ^2 $P = 0.007$), while it was not significantly different between TAb+NG and TAb–NG (5.0 vs 3.7%). However, when patients with serum TSH levels below the median value (0.90 μ U/ml) were considered, no significant difference in PTC frequency was found between nodular-HT, TAb+NG and TAb–NG (16/339, 4.7%, 35/814, 4.3%, and 58/1932, 3.0% respectively) (Fig. 2, panel B).

Discussion

In this work, we intended to approach the issue of the possible association between HT and PTC in a large series of patients with a clinical diagnosis of nodular-HT, assessing the diagnosis of PTC by cytology. The reliability of our cytological exam was validated in a large series of patients in whom histology was available (Rago *et al.* 2010), the rate of false positive and false negative exam being 1.2 and 1.8% respectively. In this study, we have further corroborated these data, showing that the cytological diagnosis of PTC was confirmed on histology in 98.3% of patients. The evaluation of this large series of patients submitted to cytological exam allowed to avoid the obvious selection bias of histological series that do not include patients with small NG with benign cytology. In our series, nodular-HT patients with PTC were younger and presented a higher female/male rate compared with patients with NG, most likely as a consequence of the different prevalence of these two thyroid diseases independently of the presence of PTC. No significant difference was observed in frequency of

the different histological variants of PTC and the size of primary tumor in relation with the clinical diagnosis. Interestingly, the frequency of node metastasis was significantly higher in PTC patients with a presurgical diagnosis of nodular-HT than of TAb–NG. These results are in agreement with the findings recently reported by Haymart *et al.* (2008a) who have shown a significant association between extrathyroidal extension of PTC and HT diagnosed on histology.

In the last few years, it has been reported that in patients with nodular thyroid diseases the risk of thyroid malignancy increases with serum TSH concentrations (Boelaert *et al.* 2006, Haymart *et al.* 2008a,b, Jonklaas *et al.* 2008, Polyzos *et al.* 2008, Fiore *et al.* 2009a, Jin *et al.* 2010). Recently, we have shown that thyroid autonomy (Fiore *et al.* 2009a) and treatment with L-T₄ (Fiore *et al.* 2010), by reducing TSH levels, may slow down progression of PTC and development of clinically detectable cancer. Therefore, we hypothesized that TSH may also play a role in the development of PTC in patients with nodular-HT, hypothyroidism being the hallmark of the disease.

The results reported in this paper show that the frequency of PTC in nodular-HT patients was significantly higher compared with patients with NG and was in strong relationship with TSH levels, confirming the data already reported in the literature for NG (Boelaert *et al.* 2006, Haymart *et al.* 2008a, Jonklaas *et al.* 2008, Polyzos *et al.* 2008, Fiore *et al.* 2009a). We are aware that the diagnosis of HT may be cumbersome on clinical grounds. The clinical diagnosis relies on the presence of hypothyroidism and of circulating TAb. However, low levels of TAb may be detected in patients with NG in the absence of a diffuse lymphocytic infiltration of the thyroid as already reported by us (Mariotti *et al.* 1990) and others (Knobel *et al.* 1994). For this reason, it is not possible to avoid an overlap between the clinical diagnosis of nodular-HT and of NG in euthyroid patients with nodular thyroid disease and positive serum TAb. In this

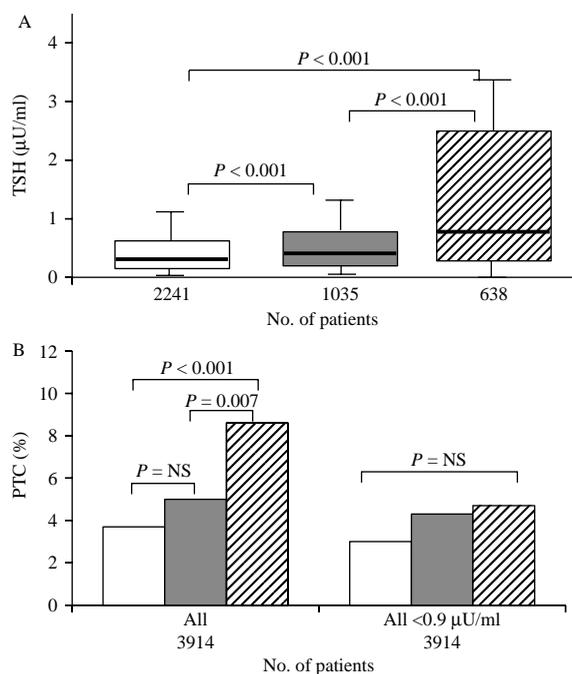


Figure 2 (Panel A) Box-whisker plots of TSH levels ($\mu\text{U/ml}$) in L-T_4 -treated patients with TAB-NG (white column), TAB+NG (gray column), and nodular-HT (striped column). Results are reported as median values (black lines), interquartile (25th–75th percentiles) range (boxes) and 10th–90th percentiles (whiskers). The statistical difference between groups was evaluated by the Mann-Whitney U test. TSH levels were significantly higher in nodular-HT compared with both TAB-NG and TAB+NG; significantly higher TSH levels were present in TAB+NG compared with TAB-NG patients (P value reported in figure). (Panel B) Frequency of PTC in L-T_4 -treated patients with TAB-NG (white columns), TAB+NG (gray columns), and nodular-HT (striped columns). In the whole series of L-T_4 -treated patients a higher frequency of PTC was observed in nodular-HT compared with both TAB-NG and TAB+NG; a higher but not significantly different frequency of PTC was observed in TAB+NG compared with TAB-NG patients. In patients with serum TSH levels below the median value ($0.90 \mu\text{U/ml}$), no significant difference in PTC frequency was found between nodular-HT, TAB-NG and TAB+NG (χ^2 P value reported in figure).

work, patients with NG and no sign of humoral thyroid autoimmunity were included in the TAB-NG group. The clinical diagnosis of nodular-HT was performed in patients who were hypothyroid, in those who had high serum TAB (i.e. both TgAb and TPOAb higher than 100 U/ml) and in 133 patients who were euthyroid, had low serum TAB, but had a clear hypoechoic ‘thyroiditis’ pattern at thyroid ultrasound. In this last group the diagnosis of nodular-HT was made because it has been shown that the typical hypoechoic pattern at ultrasound is predictive of the development of hypothyroidism in patients with TAB (Marcocci et al. 1991, Vejbjerg et al. 2006). On the other hand, euthyroid patients with NG, positive serum TAB, and no sign of thyroiditis at ultrasound were included in the

TAB+NG group. We are aware that the clinical diagnoses of nodular-HT and of TAB+NG may be questionable. For this reason, we examined the prevalence of PTC in the whole series of untreated patients taking into account only an objective parameter, i.e. the serum titer of TAB. The frequency of PTC was significantly higher (9.3%) in patients with high TAB levels (all included in the nodular-HT group) compared with both patients with lower levels of serum of TAB (6.8%) and negative TAB (6.4%). Accordingly, patients with high titers of TAB also had higher TSH levels with respect to patients with lower levels or negative serum TAB. Thus, even not taking into account the clinical diagnosis, the two factors associated with a higher frequency of PTC were serum TAB and serum TSH.

The question at this point was whether autoimmunity *per se* or rather autoimmunity as a cause of increased serum TSH is responsible for the increased frequency of PTC. A multivariate analysis was performed to identify the variables independently associated with an increased probability of PTC. According to a forward stepwise binary logistic regression model serum TSH was the most significant factor associated with PTC (OR 1.111, per each increase of $1 \mu\text{U/ml}$, $P < 0.01$). Interestingly, TPOAb were unrelated with PTC, as already shown by Boelaert et al. (2006), while TgAb levels showed a slight association with PTC (OR 1.001, per each increase of 1 U/ml , $P = 0.01$). A similar finding has recently been reported by Kim et al. (2010). This result is puzzling, as both TgAb and TPOAb are hallmarks of thyroid autoimmunity, full-blown HT being more frequently associated with high titer of TPOAb than of TgAb (Mariotti et al. 1990). However, it should be considered that in PTC patients, circulating TgAb can either be the expression of a coexistent HT or a reaction to a structural modification of thyroglobulin as a result of the neoplastic process. In agreement with this hypothesis it has already been shown that TgAb recognize different epitopes of thyroglobulin in patients with PTC or with HT (Latrofa et al. 2008). Our data, showing a slight but significant association of PTC with TgAb and not with TPOAb, suggest that in PTC patients TgAb may be the result of an immune reaction to an antigenically modified thyroglobulin, rather than the expression of thyroid autoimmunity.

The pivotal role of serum TSH has also been confirmed evaluating the relationship between TSH levels and frequency of PTC in a large series of patients under treatment with L-T_4 . This therapy was inhomogeneous in the patients included in this study, the

purpose of L-T₄ treatment being to reduce TSH levels to the lower values of normal range in all patients with NG and to correct hypothyroidism bringing serum TSH within the range of normal values in more than half of patients with nodular-HT. As a result of this uneven treatment, higher levels of TSH and higher frequency of PTC were observed in nodular-HT with respect to NG in the whole series of L-T₄-treated patients. However, when only patients with serum TSH levels below the median value (0.90 µU/ml) were taken into account, no significant difference in PTC frequency was found between nodular-HT, TAB–NG and TAB+NG. This observation suggests that in nodular-HT patients treatment with L-T₄, which reduces TSH levels, may reduce the frequency of PTC, as already reported in patients with NG (Fiore *et al.* 2010). We need to underscore that this is a cross-sectional study that includes all patients at their first observation. In this study, it was not possible to establish how long the patients had been treated with L-T₄ and how long and how persistently their TSH levels had been low. The analysis of the clinical records of several of these patients indicated that they have been under treatment with L-T₄ for a period ranging from 1 to 10 years, but it was not possible to know the TSH levels for the entire length of L-T₄ treatment. On the other hand, all the studies reported in the literature on the relation between serum TSH and frequency of PTC are cross sectional and do not deal with the problem of TSH level in time. Longitudinal case–control studies are required to address this question.

We hypothesize that higher TSH levels increase the probability that mutated oncogenes may cause cancer clinically detectable. PTC frequently has genetic alterations leading to the activation of the mitogen-activated protein kinase signaling pathway. Most common mutations in PTC are point mutations of the *BRAF* gene and RET/PTC rearrangements. These genetic alterations are found in more than 70% of PTC and they rarely overlap in the same tumor (Ciampi & Nikiforov 2007, Nikiforova & Nikiforov 2008). Our data indicate that PTC needs TSH to progress and become clinically evident and we have already shown that the development of thyroid autonomy, reducing TSH levels, may slow down cancer progression reducing the frequency of clinically detectable cancer (Fiore *et al.* 2009a). Our data do not allow drawing any conclusion on the possible involvement of TSH in the induction of such mutations. In a recent work of Franco *et al.* (2011) the authors have shown that mice with a thyroid-specific knockin of oncogenic *Braf* present invasive thyroid cancer and have high TSH levels.

Interestingly, when they were crossed with TSH-receptor knockout mice, *Braf* mutated gene was not able to induce cancer. Furthermore, in mice with wild-type TSH-receptor suppression of TSH did not revert the phenotype once tumors were established, suggesting a key role of TSH signaling in the initiation of *Braf*-induced PTC.

In conclusion, our results show that nodular HT is associated with a significant increase in the frequency of PTC and that the increased levels of serum TSH, a consequence of the thyroid damage by the autoimmune process, is the main responsible factor for this association. Furthermore, this study suggests that there may be a role for L-T₄ treatment in decreasing cancer risk in patients with nodular-HT.

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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