Lower levels of TSH are associated with a lower risk of papillary thyroid cancer in patients with thyroid nodular disease: thyroid autonomy may play a protective role

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Abstract

Higher TSH values, even within normal ranges, have been associated with a greater risk of thyroid malignancy. The relationship between TSH and papillary thyroid cancer (PTC) has been analyzed in 10 178 patients submitted to fine needle aspiration of thyroid nodules with a cytology of PTC ($n=497$) or benign thyroid nodular disease (BTND, $n=9681$). In 942 patients, submitted to surgery (521 from BTND and 421 from PTC), the histological diagnosis confirmed an elevated specificity (99.6%) and sensitivity (98.1%) of cytology. TSH levels were significantly higher in PTC than in BTND both in the cytological and histological series and also in patients with a clinical diagnosis of multinodular goiter (MNG) and single/isolate nodule (S/I). A significant age-dependent development of thyroid autonomy (TSH <0.4 μU/ml) was observed in patients with benign thyroid disease, but not in those with PTC, diagnosed both on cytology and histology. In patients with MNG, the frequency of thyroid autonomy was higher and the risk of PTC was lower compared to those with S/I. In all patients, the presence of thyroid auto-antibodies (TAb) was associated with a significant increase of TSH. However, both in TAb positive and TAb negative patients TSH levels were significantly higher in PTC than in BTND. Our data confirm a direct relationship between TSH levels and risk of PTC in patients with nodular thyroid diseases. Thyroid autonomy conceivably protects against the risk of PTC, while thyroid autoimmunity does not play a significant role.

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Introduction

Thyroid cancer is the most common malignant tumor of the endocrine system. In 2003, the American Cancer Society reported an incidence in the USA of 1/10 000, increasing with a rate higher than 5% per year for a decade (Davies & Welch 2006). Papillary thyroid cancer (PTC) accounts for more than 80% of all thyroid malignancies (Hundahl *et al*. 1998).

TSH is involved in the regulation of thyroid function such as secretion of thyroid hormones, maintenance of thyroid-specific gene expression (differentiation), and gland growth. Experimental studies and clinical data have demonstrated that thyroid-cell proliferation is dependent on TSH and that well-differentiated thyroid cancers usually retain responsiveness to TSH. These observations provide the rationale for TSH suppression as a treatment for differentiated thyroid cancer (Biondi *et al*. 2005). Several reports have shown that patients with well-differentiated thyroid cancers respond to TSH suppressive treatment with L-thyroxine (L-T_4), resulting in decreased disease progression, recurrence rates, and cancer-related mortality (Mazzaferri & Jhiang 1994, Mazzaferri 1999, Sipos & Mazzaferri 2008).

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Recently it has been reported that in patients with nodular thyroid diseases, the risk of malignancy increases with serum TSH concentrations and, even within normal ranges, higher TSH values are associated with a significantly greater likelihood of thyroid cancer (Boelaert *et al*. 2006, Jonklaas *et al*. 2008, Polyzos *et al*. 2008). Higher TSH levels have also been associated with advanced stage thyroid cancer and it has been suggested that TSH may play a central role in its development and progression (Haymart *et al*. 2008*b*). However, in some of these studies (Boelaert *et al*. 2006, Polyzos *et al*. 2008) different thyroid malignancies were grouped, including medullary or anaplastic cancers or thyroid lymphomas, that have never been reported to be TSH dependent. Besides, in other studies (Haymart *et al*. 2008*b*, Jonklaas *et al*. 2008) only patients submitted to thyroid surgery were included, and it is not possible to rule out a potential selection bias because histological series usually do not include patients with small benign nodular goiter.

In this work, we intended to study the relationship between TSH and PTC in a large and homogeneous series of patients subjected to fine needle aspiration (FNA), after validating the results of cytology in a subgroup of patients submitted to surgery. We also intended to analyze the relationship between PTC, TSH and the presence of serum thyroid autoantibodies (TAb). To this purpose, we ruled out possible factors that may affect TSH levels, including in the study only patients who were not on therapy with L-T_4 or methimazole. Our results indicate that the detection of higher TSH levels in PTC than in benign thyroid nodular disease (BTND) is mainly related to the development of thyroid 'autonomy' in nodular goiter, rather than thyroid autoimmunity in PTC.

Patients and methods

Patients

During the years 1997–2004, 33 774 patients underwent FNA biopsy of thyroid nodules cold at scintiscan in our department. Among these, we selected 10 178 patients (males, 2007; females, 8171; mean age 49.2 ± 13.2 years), who were included in the present study because they fulfilled the following criteria:

a) they had a diagnostic cytological exam (patients with non-diagnostic or indeterminate cytology were excluded)
b) they were not taking L-T_4 or methimazole and were not overtly hyperthyroid or hypothyroid
c) they had TSH, free thyroid hormones, and anti-thyroid antibodies measured simultaneously with FNA
d) the diagnosis of Graves' disease and Hashimoto's thyroiditis had been excluded on clinical grounds. Patients were defined as affected by nodular Hashimotos' thyroiditis if they showed a diffused hypoecoic 'thyroiditis' pattern at thyroid ultrasound and had high levels of anti-thyroglobulin (TgAb) and/or anti-thyroperoxidase (TPOAb) antibodies. The diagnosis of Graves' disease was made according to usual standard criteria including active or treated hyperthyroidism, goiter with diffused hypoecoic pattern at thyroid ultrasound, ophthalmopathy, and serum positive for anti-TSH receptor antibodies, and/or TgAb or TPOAb.

All patients gave their informed consent to the study. According to clinical findings, ultrasound examination and thyroid scintiscan patients were subdivided into two diagnostic groups. The patients with single/isolate (S/I) thyroid nodule ($n = 3577$) had a single, cold nodule in a normal or slightly enlarged thyroid gland. The multinodular goiter (MNG) patients ($n = 6601$) presented a goiter with multiple nodules at ultrasound examination. At thyroid scan they had only cold nodules or both cold and 'hot' nodules. In these patients, FNA biopsy was performed only on cold thyroid nodules.

Thyroid function tests

Serum free T_4 (FT_4) and triiodothyronine (FT_3) were measured by RIA (FT_4 by Liso-Phase kit – normal values 7–17 pg/ml; FT_3 by Liso-Phase kit – normal values -2.7–5.7 pg/ml; Technogenetics, s.r.l., Milan, Italy). Serum TSH was measured by a sensitive immunoradiometric assay (Delphia Pharmacia, Turku, Finland – normal values 0.4–3.4 μU/ml). TgAb and TPOAb were measured by an immunoenzymatic assay (AIA-Pack TgAb, and TPOAb, Tosoh, Tokyo, Japan) and expressed as U/ml. Normal values were <30 U/ml for TgAb and <10 U/ml for TPOAb.

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 British Thyroid Association (2007).

For histological diagnosis, formalin-fixed, paraffin-embedded nodular tissues were stained by hematoxylin and eosin. The diagnosis was made blindly by two independent pathologists (F B, C U), according to the World Health Organization guidelines. When the results were discordant, agreement was found by conjoint re-examination of each case. In patients with PTC, cancer was staged according to the TNM classification (Sobin 2002).

Statistical analysis

TSH values were expressed as median and interquartile range (25–75p). Non-parametric tests (χ^2, Mann–Whitney or Kruskall–Wallis) were used as appropriate and considered statistically significant where $P<0.05$.

Results

FNA cytology and histological validation

On the whole, 497/10 178 (4.9%) patients had a FNA cytology suggestive or indicative of PTC (PTC group), and 9681/10 178 (95.1%) had one or more cytological benign thyroid nodules and were included in the BTND group. The soundness of cytological diagnosis was evaluated in a sample of 942 patients submitted to surgery. This group included the majority (421/497, 85%) of patients with a cytological diagnosis of PTC and 521 patients of BTND group who had been submitted to surgery. The histological diagnosis of PTC was confirmed in 419/421 (99.5%) patients with a cytology suggestive or indicative of PTC and was found in 9/521 (1.7%) patients with cytological diagnosis of BTND. Thus, FNA cytology had an elevated specificity (99.6%) and sensitivity (98.1%). In our series, the positive predictive value of cytology was 99.5% and the negative predictive value was 98.3%.

Free thyroid hormones and TSH values in BTND and PTC patients

As shown in Table 1, in the large group of patients submitted to FNA, serum TSH levels in PTC patients (median 1.10 μU/ml; 25–75p 0.70–1.70 μU/ml) were significantly higher than in BTND (median 0.70 μU/ml; 25–75p 0.30–1.20 μU/ml). In contrast to what was observed with TSH values, serum FT_3 and FT_4 levels were not significantly different in BTND and PTC. However, serum FT_3 was significantly higher in patients with thyroid autonomy (i.e. TSH <0.4 μU/ml) with respect to those with normal TSH values, both in PTC and BTND (Table 2).

Table 1 Free thyroid hormones and TSH levels (expressed as median and interquartile range) in benign thyroid nodular disease (BTND) and papillary thyroid cancer (PTC) groups

	BTND	PTC	*P* value[a]
FT_3 (pg/ml)	3.6 (3.1–4.0)	3.6 (3.1–3.9)	NS
FT_4 (pg/ml)	10.9 (9.6–12.4)	10.9 (9.7–12.5)	NS
TSH (μU/ml)	0.70 (0.30–1.20)	1.10 (0.70–1.70)	<0.0001

[a]Mann–Whitney test.

Also in the subgroup of patients submitted to surgery, serum TSH levels were significantly higher ($P<0.001$) in PTC (median 1.10 μU/ml; 25–75p 0.70–1.80 μU/ml) than in BTND (median 0.50 μU/ml; 25–75p 0.17–0.90 μU/ml), confirming the results observed in the whole cytological series (data not shown).

Prevalence of PTC according to serum TSH levels

In the whole group of patients, 2024/10 178 (19.8%) had subclinical or overt hyperthyroidism, i.e. serum TSH concentrations below the normal range (0.4 μU/ml), and normal or elevated serum-free thyroid hormones respectively. A total of 7893 patients had serum TSH levels within the normal range. These subjects were subdivided into four quartiles of similar size according to their TSH values. A group of 261 patients with nodular thyroid disease had serum TSH levels slightly higher than normal, ranging from 3.5 to 10 μU/ml. Anti-thyroid antibodies were detected in 183/261 (70.1%) of these patients who conceivably had an autoimmune thyroiditis, even in the absence of

Table 2 Free triiodothyronine (FT_3; pg/ml) levels (expressed as median and interquartile range) in benign thyroid nodular disease (BTND) and papillary thyroid cancer (PTC) groups in patients with TSH $<$ or ≥ 0.4 μU/ml. The number of patients (*n*) in each group is indicated

	TSH <0.4 μU/ml (*n*=2024)	TSH ≥0.4 μU/ml (*n*=8154)	*P* value[a]
BTND (*n*=9681)	3.9 (3.4–4.6) (*n*=1979)	3.5 (3.1–3.9) (*n*=7702)	<0.0001
PTC (*n*=497)	3.9 (3.5–4.8) (*n*=45)	3.6 (3.1–3.4) (*n*=452)	<0.0001

[a]Mann–Whitney test.

the characteristic hypoecoic pattern at ultrasound examination. The prevalence of PTC according to serum TSH concentrations is shown in Fig. 1. The frequency of PTC was higher in subjects with higher TSH values, being the lowest in patients with subnormal TSH values (51/2024; 2.5%) and the highest in patients with TSH values between 1.6 and 3.4 μU/ml (152/1665; 9.1%). It is worth underlining that in patients with TSH between 3.5 and 10 μU/ml the frequency of PTC (21/261; 8.0%) was not significantly different with respect to patients with TSH in the upper limit of normal range (χ^2 test $P=0.1$). The odd ratio and 95% confidence interval of PTC according to TSH levels are reported in Table 3.

TSH value in BTND and PTC patients according to age

As shown in Fig. 2, panel A, serum TSH levels showed a significant reduction with age (Kruskall–Wallis, $P<0.0001$), as expected in patients with nodular thyroid disease. When TSH levels according to age were analyzed separately in PTC and BTND (Fig. 2, panel B), TSH was significantly higher in PTC than BTND in all age groups. Interestingly, BTND patients showed a significant, age-dependent reduction of TSH values (Kruskall–Wallis, $P<0.0001$), while in PTC the reduction of TSH in older patients was less evident and only slightly significant (Kruskall–Wallis, $P=0.03$).

Thyroid functional autonomy, defined as serum TSH levels below the lower limit of the normal range (0.4 μU/ml), was found in 1973/9681 (20.4%) BTND and 51/497 (10.3%) PTC (χ^2, $P<0.0001$). While in PTC the frequency of thyroid autonomy showed no age-dependent distribution, in BTND it was progressively increasing with age and was significantly higher than PTC in all classes of age with the exception of younger subjects (Fig. 3, panel A). In patients submitted to surgery, the age-dependent thyroid autonomy presented a similar pattern, showing a significant increase in older patients only in the BTND group (Fig. 3, panel B). This finding confirmed the results observed in the whole cytological series.

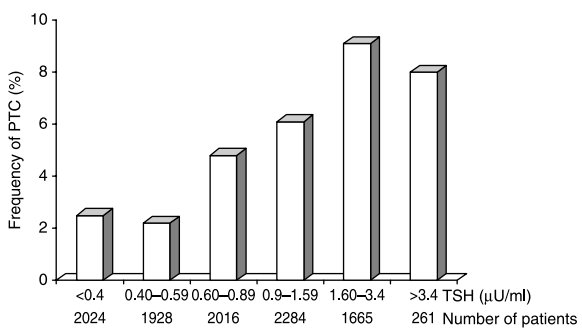

Figure 1 Prevalence of PTC according to serum TSH concentrations. Patients with serum TSH level within the normal range were divided into four quartiles of similar size according to their TSH values. Patients with serum TSH levels lower (0.4 μU/ml) or higher (from 3.5 to 10 μU/ml) than the normal range values were considered separately. The odd ratio and 95% confidence interval of PTC according to TSH levels are reported in Table 3.

Table 3 Odd ratio (OR) and 95% confidence interval (CI) of papillary thyroid cancer (PTC) according to TSH levels

TSH (μU/ml)	OR	95% CI	*P* value[a]
0.40–0.59	0.80	0.51–1.27	0.18
0.60–0.89	2.01	1.46–2.77	<0.0001
0.90–1.59	2.66	1.98–3.58	<0.0001
1.60–3.40	4.29	3.17–5.08	<0.0001
>3.40	3.50	2.10–5.83	0.0011

[a]Binary logistic regression analysis.

Risk of PTC according to clinical diagnosis and thyroid autonomy

The overall risk of PTC was significantly lower in MNG than in S/I both in the whole group (234/6601, 3.5% vs 263/3577, 7.3%; χ^2, $P<0.0001$) and in the subgroups of patients classified according to the presence of thyroid autonomy (22/1595, 1.4% vs 23/429, 5.4%; χ^2, $P<0.0001$) or its absence (212/5006, 4.2% vs 240/3148, 7.6%; χ^2, $P<0.0001$) (Fig. 4, panel A). In patients with MNG the risk of PTC was much higher in those with TSH ≥0.4 μU/ml (212/5006, 4.2%) than in those with thyroid autonomy (22/1595, 1.4%; χ^2, $P<0.0001$.), while in patients with S/I this difference did not reach statistical significance (240/3148, 7.6% vs 23/429, 5.4%; χ^2, $P=0.09$) (Fig. 4, panel A).

As expected, thyroid autonomy was much more frequent in patients with MNG (1595/6601, 24.1%) than those with S/I (429/3577, 11.9%; χ^2, $P<0.0001$, not shown). In patients with MNG thyroid autonomy was much more frequent in patients with the cytological diagnosis of BTND (1573/6367, 24.7%) than in those with PTC (22/234, 9.4%; χ^2, $P<0.0001$), while in S/I the prevalence of thyroid autonomy was not significantly different between patients with benign (406/3314, 12.2%) and malignant cytology (23/263, 8.7%; χ^2, $P=0.09$) (Fig. 4, panel B).

TSH values and PTC stage

Patients with PTC were grouped according to TNM and results are shown in Fig. 5. TSH values in patients with stage T3–T4 ($n=170/497$, 34.2% median

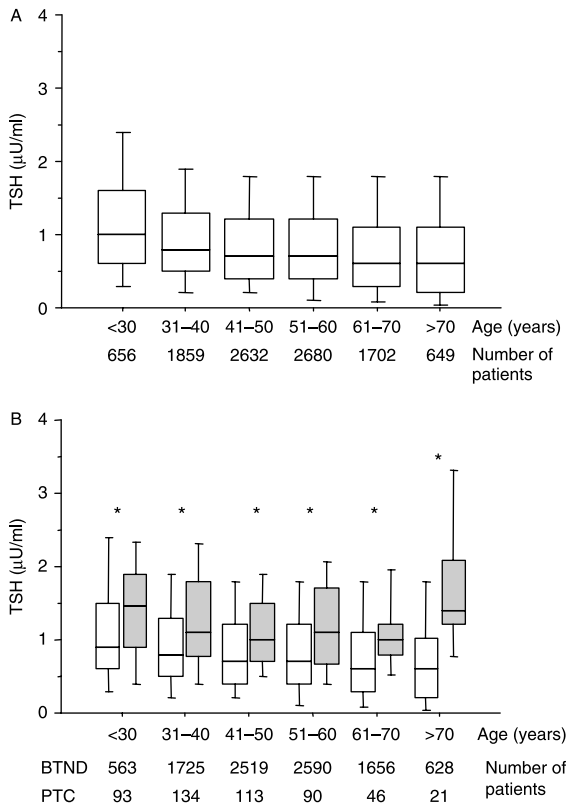

Figure 2 Box-whisker plots illustrating median, quartiles, and ranges of TSH levels according to age. As shown in panel A, serum TSH levels show a significant reduction with age (Kruskall–Wallis test, $P<0.0001$). The number of patients included in each age group is indicated in the bottom of the figure. When BTND (white box) and PTC (gray box) patients were grouped according to age (B), TSH was significantly higher in PTC than in BTND in all age groups ($*=\chi^2$, $P<0.01$). Besides, BTND patients showed a significant, age-dependent reduction of TSH values (Kruskall–Wallis test, $P<0.0001$), while in PTC the reduction of TSH in older patients was less evident and only slightly significant (Kruskall–Wallis test, $P=0.03$). The number of patients with BTND and PTC in each age group is indicated in the bottom of the figure.

TSH 1.30 μU/ml; 25–75p 0.85–1.95 μU/ml) were significantly higher (Mann–Whitney, $P=0.001$) than in patients with stage T1–T2 ($n=327/497$, 65.8% median TSH 1.00 μU/ml; 25–75p 0.60–1.60 μU/ml). When patients were divided according to the presence or the absence of neck node metastasis, TSH values in patients with N1 ($n=159/497$, 32.0%, median TSH 1.40 μU/ml; 25–75p 0.90–1.90 μU/ml) were significantly higher (Mann–Whitney, $P=0.002$) than in patients with N0 ($n=338/497$, 68.0%, median TSH 1.00 μU/ml; 25–75p 0.60–1.70 μU/ml). Distant metastasis were detected only in 5/497 (1%) patients and because of this low number TSH values in M1 versus M0 patients were not analyzed.

TSH value in BTND and PTC patients according to the presence of TAb

To address the question of the possible effect of thyroid autoimmunity on TSH levels, we also analyzed TSH levels according to the presence of TAbs. In TAb positive patients TSH (0.70 μU/ml; 25–75p 0.30–1.20 μU/ml) was significantly higher (Mann–Whitney, $P=0.002$) than in TAb negative subjects (0.70 μU/ml; 25–75p 0.30–1.30 μU/ml). It is worth pointing out that, even if median TSH levels were identical in both in TAb positive and negative patients, the TSH values in the upper quartile were higher in TAb positive than TAb negative subjects, accounting for the significant statistical difference observed between these two groups.

Serum TSH levels were significantly higher in PTC than in BTND both in TAb positive and negative patients (Fig. 6). In TAb positive patients the median

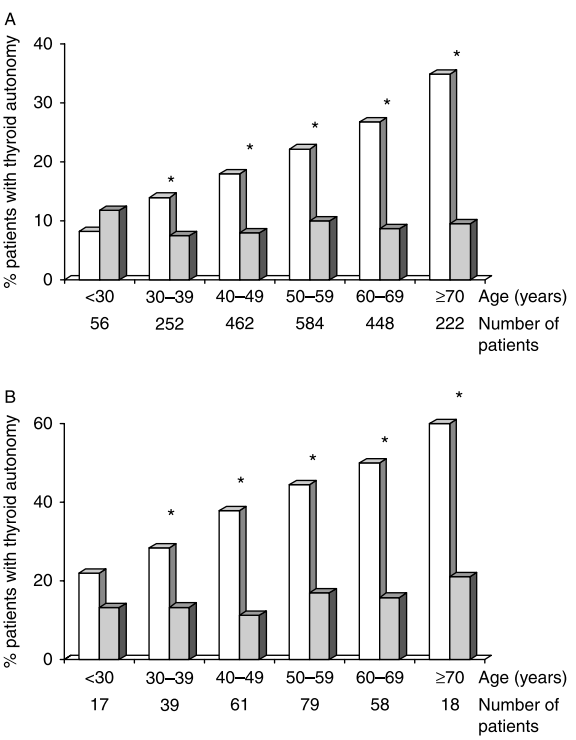

Figure 3 Frequency of thyroid autonomy in PTC (gray bars) and BTND (white bars) according to age in patients with thyroid autonomy. In BTND patients, a progressive, age-dependent increase of thyroid autonomy was observed, while no age-dependent distribution was present in PTC patients. With the exception of younger subjects, the frequency of thyroid autonomy was significantly higher in BTND than PTC in all classes of age ($*=\chi^2$, $P<0.01$). Similar results were observed both in the whole cytological series (panel A) and in the subgroup of patients submitted to surgery (panel B). The number of patients with thyroid autonomy (BTND+PTC) in each age group is reported in the bottom of the figure. The total number of patients in each age group is indicated in Fig. 2.

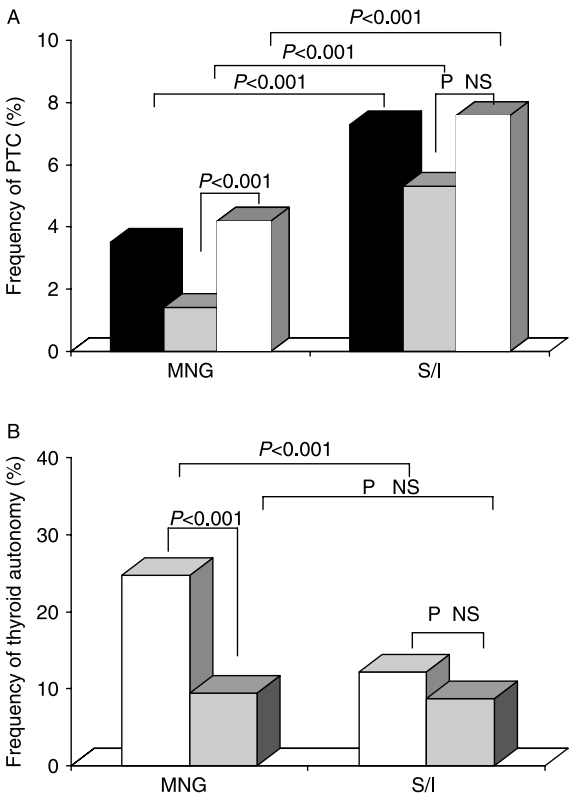

Figure 4 Panel A, frequency of PTC in the whole groups of patients with MNG and S/I (black bars) and in the same patients subdivided according to the presence (gray bars) or the absence (white bars) of thyroid autonomy. Panel B, frequency of thyroid autonomy according to clinical diagnosis (MNG and S/I) in patients with BTND (white bars) and those with PTC (gray bars). The statistical differences between the groups (χ^2 and *P* values) are reported.

level of TSH was 1.20 μU/ml (25–75p 0.70–1.80 μU/ml) in PTC and 0.70 μU/ml (25–75p 0.30–1.30 μU/ml) in BTND (Mann–Whitney, $P<0.0001$). In TAb negative patients the median level of TSH was 1.10 μU/ml (25–75p 0.70–1.70 μU/ml) in PTC and 0.70 μU/ml (25–75p 0.30–1.11 μU/ml) in BTND (Mann–Whitney, $P<0.0001$). The frequency of PTC was not significantly different (χ^2, $P=0.21$ NS) between TAb negative (300/5593, 5.1%) and TAb positive (197/4205, 4.6%) patients.

Discussion

Well-differentiated thyroid cancer usually retains responsiveness to TSH and for this reason TSH suppression therapy with L-T_4 plays an important role in its treatment (Mazzaferri & Young 1981, Mazzaferri 1991, 1999, Mazzaferri & Jhiang 1994, Biondi *et al.* 2005, Sipos & Mazzaferri 2008). In this work, we analyzed the relationship between serum TSH levels and risk of PTC in a large series of patients subjected to FNA cytology of thyroid nodules. We decided to focus only on PTC because is the most frequent thyroid cancer, accounting for more than 80% of all thyroid malignancies and, as is follicular thyroid cancer, is TSH dependent. Furthermore, the cytological diagnosis of PTC is usually highly dependable, while the diagnosis of follicular thyroid cancer requires histological examination in order to evaluate infiltration of the tumor capsule or vessels. We validated the reliability of cytological exam in a subgroup of patients submitted to surgery and in our series the positive and negative predictive values of cytology were very high, being 99.5 and 98.3% respectively.

After confirming the soundness of cytology, we studied a large series of untreated patients with thyroid nodules subjected to FNA, only including subjects with a well-defined cytological diagnosis (patients with non-diagnostic or indeterminate cytology were

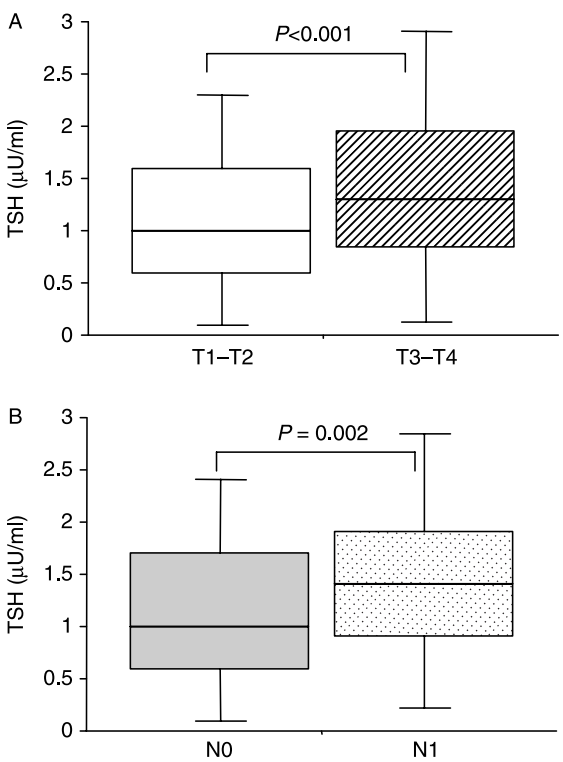

Figure 5 Box-whisker plot illustrating median, quartiles, and ranges of TSH levels in PTC according to TNM. Panel A: TSH levels in patients with stage T3–T4 (striped box) were significantly higher (Mann–Whitney test $P<0.001$) than in stage T1–T2 (white box). Panel B: TSH values in patients with N1 (dotted columns) were significantly higher (Mann–Whitney $P=0.002$) than in patients with N0 (gray columns).

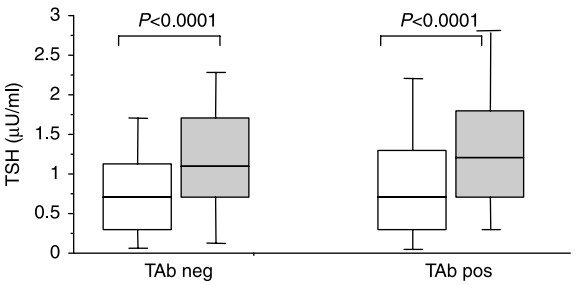

Figure 6 Box-whisker plot illustrating median, quartiles, and ranges of TSH levels according to the presence of anti-thyroid antibodies (TAb). TSH levels in PTC (gray box) were significantly higher than in BTND (white box) both in TAb positive patients and in subjects with no detectable TAb (Mann–Whitney test, $P<0.01$).

excluded). By including a selection of patients, these inclusion criteria allowed us to evaluate the relationship between TSH levels and thyroid cancer in a large and homogeneous cytological series, avoiding the obvious selection bias of histological series that do not include patients with small nodular goiter. Besides, we ruled out possible factors that may affect TSH levels, by including only patients who were not on therapy with L-T_4 or methimazole and patients who were not affected by Hashimoto's thyroiditis and Graves' disease, diagnosed on clinical grounds. Even though these inclusion criteria determined a selection of patients, this procedure must be carried out in order to exclude both treatments and thyroid diseases that may affect TSH levels.

Our results demonstrate that TSH levels are slightly, but significantly higher in PTC compared to benign thyroid diseases and that the prevalence of PTC increases with TSH, being the highest in patients with serum TSH in the upper limit of the normal range. In agreement with these observations, we found a significantly higher TSH level in PTC compared with BTNDs in a subgroup of patients submitted to surgery, in whom the cytological diagnosis had been validated by histology. These findings confirm the results reported by Boelaert *et al*. (2006), who found an increased risk of thyroid malignancy in patients with higher TSH levels. However, in this study different thyroid cancers were considered together, including thyroid lymphomas and medullary thyroid cancers which are not supposed to be TSH dependent. Besides, only 20 patients with thyroid malignancy were diagnosed by cytology and the majority of thyroid cancers were detected on histology, representing a possible selection bias. On the other hand, our results were obtained in a large cytological series and were confirmed in a wide subgroup of patients submitted to surgery. An increased risk of differentiated thyroid cancer in patients with higher TSH has been reported also by Haymart *et al*. (2008*b*). In this work, only a histological series has been evaluated and the possibility of bias cannot be ruled out, as the prevalence of thyroid cancer in this series (241/843 = 28.1%) quite high compared with the frequency of differentiated thyroid cancer in patients with nodular thyroid disease.

In the whole group of patients included in the present study, serum TSH levels decrease progressively with age. This finding has already been observed in iodine deficient areas, such as those the majority of our patients came from, where longstanding iodine deficiency causes higher frequency of thyroid nodularity and autonomy in older people (Fenzi *et al*. 1985, Vitti *et al*. 1990, Aghini-Lombardi *et al*. 1999). When patients were classified as affected by benign thyroid disease or PTC according to the results of cytology, the age-dependent reduction of TSH levels was highly significant in the first group, while in patients with PTC this phenomenon was much less evident. Indeed, the TSH levels in patients with PTC were close to the age-specific distribution of TSH found in people living in iodine sufficient areas, as reported in the NHANES III survey (Hollowell *et al*. 2002, Aoki *et al*. 2007, Surks & Hollowell 2007). These data strongly suggest that in our series of patients, the higher levels of TSH in PTC with respect to BTND are not due to an increase of thyrotropin in patients with thyroid cancer, but are mainly related to the reduction of serum TSH in patients with nodular goiter. In agreement with this hypothesis a significant age-dependent development of thyroid autonomy (TSH below 0.4 μU/ml) was observed in BTNDs, but not in PTC. When patients were considered separately, according to their clinical diagnosis, in patients with benign thyroid disease the prevalence of thyroid autonomy was significantly higher in MNG than in S/I (Fig. 4A). In MNG, thyroid autonomy seems to play a protective role in the development of PTC, the prevalence of PTC in patients with TSH <0.4 μU/ml being significantly lower than in patients with no evidence of thyroid autonomy ($P<0.0001$). On the other hand, in S/I the frequency of PTC in patients with thyroid autonomy was lower (5.3%) with respect to those with normal TSH levels (7.6%), but this difference did not reach statistical significance (χ^2, $P=0.09$). However, the relatively small number of patients with PTC and thyroid autonomy in the S/I group may account for the lack of statistical significance. Further studies are needed to clarify this point. In this regard, Ngan *et al*. (2009) have

found germline mutations of the *TTF1* gene in a subgroup of patients with MNG, and that mutations of this gene are not present in patients without history of MNG and in healthy subjects. In addition, Gudmundsson *et al*. (2009) have shown that variants on specific loci (9q22.33 and 14q13.3) are associated with increased risk of papillary and follicular thyroid cancer and both alleles are associated with lower TSH levels in the general population. Interestingly, the gene located at the 14q13.3 locus is TTF1. These observations suggest that a different genetic background may be present in PTC patients with MNG and that specific mutations may allow PTC to growth in patients with low serum TSH levels. In our study group the prevalence of PTC in patients with serum TSH <0.4 μU/ml was significantly lower than in patients with no evidence of thyroid autonomy in MNG, but our data did not allow us to draw a definitive conclusion in patients with S/I nodules. We hypothesize that the development of thyroid autonomy, by reducing TSH levels, reduces the probability that mutated oncogenes may cause cancer clinically detectable. Most common mutations in papillary carcinomas are point mutations of the *BRAF* gene and RET/PTC rearrangement. These genetic alterations are found in more than 70% of papillary carcinomas and they rarely overlap in the same tumor (Ciampi & Nikiforov 2007, Nikiforova & Nikiforov 2008). It is also widely recognized that PTC needs TSH to progress and become clinically evident. In this respect, it is important to underscore that the medical treatment of differentiated thyroid cancer has been based on the use of L-T_4 to reduce serum TSH levels (Mazzaferri & Jhiang 1994, Mazzaferri 1999, Sipos & Mazzaferri 2008). It is thus possible to hypothesize that the development of thyroid autonomy, by reducing TSH levels, may represent a form of 'self-treatment', at least in patients living in relatively iodine deficient areas such as those considered in the present study. This phenomenon may be less relevant in iodine sufficient areas.

The hypothesis that TSH is involved in the progression of PTC is further supported by the observation that higher TSH levels are associated with a more advanced cancer stage, as reported by Haymart *et al*. (2008*b*). In our series, in agreement with the results reported by Haymart *et al*. when patients with PTC were grouped according to TNM, significantly higher TSH values were observed in patients in T3–T4 with respect to those in T1–T2. A similar finding was present in patients with neck node metastasis with respect to those with no evidence of node metastasis.

In this work, we have also analyzed the relationship between the presence of humoral thyroid autoimmunity and TSH levels in patients with PTC. The relationship between thyroid autoimmunity and PTC has been suggested in many studies, but its meaning is still uncertain (Dailey *et al*. 1955, Hirabayashi & Lindsay 1965, Holm *et al*. 1985, Walker & Paloyan 1990, Baker 1995, Okayasu *et al*. 1995). Our data show that TSH is significantly higher in TAb positive than in TAb negative patients, suggesting that TAb positivity is the expression of a mild autoimmunity that may affect thyroid function. Interestingly, Haymart *et al*. (2008*a*), studying a large histological series of patients with nodular thyroid diseases submitted to surgery, have reported a significant association between pathologic Hashimoto's thyroiditis and higher TSH levels. In this work, pathologic Hashimoto's thyroiditis was detected in 20.6% of patients with DTC and 19.8% of patients with benign thyroid diseases ($P=0.4$). In our series of patients, the frequency of PTC was not significantly different between TAb positive and TAb negative patients and serum TSH levels were significantly higher in PTC than BTND both in TAb positive and negative patients. This result supports the hypothesis that humoral thyroid autoimmunity does not play a significant role in the development of thyroid cancer.

In conclusion, we have confirmed that a significant difference between serum TSH levels is present in patients with PTC and BTNDs. We have shown that this difference is mainly due to the reduction of TSH observed in patients with nodular goiter and is not related to thyroid autoimmunity. We hypothesize that the development of thyroid autonomy may account for the lower TSH levels in patients with goiter, while in PTC serum TSH levels are closer to the value of thyroid disease free population. We suggest that the possible mechanism underling these clinical observations is that thyroid autonomy, by reducing TSH levels, may slow down cancer progression and reduce the probability that mutated oncogenes may cause clinically detectable cancer. These observations may be relevant for the inclusion of thyroid function as one of the clinical parameters to consider in the evaluation of the risk of PTC in patients with nodular thyroid diseases.

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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