L-thyroxine-treated patients with nodular goiter have lower serum TSH and lower frequency of papillary thyroid cancer: results of a cross-sectional study on 27 914 patients

E Fiore[1], T Rago[1], M A Provenzale[1], M Scutari[1], C Ugolini[2], F Basolo[2], G Di Coscio[3], P Miccoli[4], L Grasso[1], A Pinchera[1] and P Vitti[1]

[1]Department of Endocrinology, [2]Section of Pathology, [3]Section of Cytopathology and [4]Department of Surgery, University of Pisa, Via Paradisa 2, 56100 Pisa, Italy

(Correspondence should be addressed to E Fiore; Email: e.fiore@ao-pisa.toscana.it)

Abstract

The risk of papillary thyroid cancer (PTC) is related to serum TSH, and the development of thyroid autonomy by reducing TSH levels decreases the frequency of PTC in patients with nodular goiter. Our aim was to investigate the effect of L-thyroxine (L-T_4) on the frequency of PTC diagnosed by cytology in a large series of patients with nodular goiter untreated ($n=20\,055$) or treated with L-T_4 ($n=7859$). L-T_4-treated patients with respect to untreated patients presented significantly lower serum TSH (median, interquartile range: 0.30 μU/ml, 0.08–0.62 μU/ml versus 0.70 μU/ml, 0.38–1.14 μU/ml; $P<0.0001$) and prevalence of PTC (3.2 vs 5.1%; $P<0.0001$). The frequency of PTC was closely related to serum TSH, with it being lowest in patients with TSH below the normal range (<0.4 μU/ml; 189/10 059, 1.9%) and highest in patients with TSH above the normal range (>3.4 μU/ml; 21/127, 16.5%), also showing a progressive increase from the lower to the upper quartile of normal range. A significantly higher proportion of L-T_4-treated patients (6650/7859, 84.6%) had serum TSH below the median (0.90 μU/ml) with respect to untreated patients (12 599/20 055, 62.8%; χ^2 *P* value <0.0001), with it being included in the range of TSH associated with a lower frequency of PTC. The relationship between serum TSH and frequency of PTC was unrelated to the type of nodularity (solitary versus multinodular) and was not age dependent. In conclusion, patients with nodular goiter, treatment with L-T_4 is responsible for the reduction of serum TSH and is associated with a decreased frequency of PTC.

Endocrine-Related Cancer (2010) **17** 231–239

Introduction

Thyroid-stimulating hormone (TSH) is the main regulator of thyroid function and is involved in the secretion of thyroid hormones, maintenance of thyroid-specific gene expression (differentiation), and thyroid cell proliferation. Serum TSH is a well-established growth factor for thyroid nodules, and the administration of L-thyroxine (L-T_4) is a common medical treatment for nodular goiter. Suppression of serum TSH concentrations by administering exogenous thyroid hormone may interfere with the growth of established nodules as well as with the formation of new thyroid nodules (Papini *et al*. 1998), even if the real effectiveness of L-T_4 treatment is still debated (Hegedus *et al*. 2003, Filetti *et al*. 2006). L-T_4 treatment also plays a pivotal role in the management of well-differentiated thyroid cancers, including papillary thyroid cancer (PTC) and follicular thyroid cancer. Well-differentiated thyroid cancers usually retain responsiveness to TSH, and this observation provides the rationale for TSH suppression as a treatment for these cancers (Biondi *et al*. 2005). Several reports have shown that patients with well-differentiated thyroid cancers take advantage of TSH-suppressive treatment with L-T_4, presenting a

1351–0088/10/017–231
DOI: 10.1677/ERC-09-0251
Online version via http://www.endocrinology-journals.org

decreased disease progression, recurrence rates, and cancer-related mortality (Mazzaferri & Jhiang 1994, Mazzaferri 1999, Sipos & Mazzaferri 2008).

It has been reported that in patients with nodular thyroid diseases, the risk of thyroid malignancy increases with serum TSH concentrations, and that even within normal ranges, higher TSH values are associated with a higher frequency and more advanced stage of thyroid cancer (Boelaert *et al*. 2006, Haymart *et al*. 2008*a*,*b*, Jonklaas *et al*. 2008, Polyzos *et al*. 2008). Recently, we have confirmed the direct relationship between TSH levels and frequency of PTC in patients with nodular thyroid disease and have shown that this association is mainly due to the development of thyroid autonomy in patients with nodular goiter, suggesting that thyroid autonomy by reducing TSH levels may slow down cancer progression (Fiore *et al*. 2009).

In the past, experiments on various animals (Fortner *et al*. 1960, Schaller & Stevenson 1966) indicated that overstimulation of the thyroid by TSH leads to hyperplasia and eventually to the development of cancer (Wynford-Thomas 1993, Farid *et al*. 1994, Feldt-Rasmussen 2001). Furthermore, Goldberg *et al*. (1964) showed that TSH suppression in rats exposed to radioiodine prevents the formation of thyroid nodules and thyroid cancers. In humans, there are no data on the protective effects of TSH suppression with L-T_4 treatment on thyroid cancer development.

In this study, our aim was to investigate the possible relationship between L-T_4 treatment and risk of PTC in patients with nodular thyroid disease. We therefore evaluated the frequency of PTC in a large series of patients subjected to fine needle aspiration (FNA) of thyroid nodules, who were not under L-T_4 treatment or were treated with L-T_4 when FNA was performed. Our results confirm a direct relationship between TSH levels and prevalence of PTC, showing a lower frequency of PTC in patients with lower TSH levels due to both the development of thyroid autonomy and the L-T_4 treatment. A significantly higher proportion of L-T_4-treated patients with respect to untreated patients had low serum TSH, and this different frequency distribution was responsible for the lower risk of PTC in patients under L-T_4 treatment.

Patients and methods

Patients

In this study, we included patients at their first observation in our Department between 1997 and 2009, who underwent FNA of cold thyroid nodules and who fulfilled the following criteria:

a) had undergone a diagnostic cytological examination (patients with nondiagnostic or indeterminate cytology were excluded),
b) had TSH, free thyroid hormones, and serum anti-thyroid antibodies measured simultaneously with FNA,
c) the diagnosis of Graves' disease and of Hashimoto's thyroiditis had been excluded on clinical grounds. In particular, patients were defined as affected by nodular Hashimoto's thyroiditis if they had positive serum anti-thyroglobulin (TgAb) and/or anti-thyroperoxidase (TPOAb) antibodies and were hypothyroid (treated or untreated) or euthyroid with a diffuse hypoechoic 'thyroiditis' pattern during thyroid ultrasound. The diagnosis of Graves' disease was made according to the standard criteria including active or treated hyperthyroidism, goiter with a diffuse hypoechoic 'thyroiditis' pattern during thyroid ultrasound, ophthalmopathy, and positive serum anti-TSH receptor antibodies (TRAbs) and/or TgAbs or TPOAbs.

All patients gave their informed consent to the study.

In this study, 27 914 patients (males: 5249, females: 22 665, mean age: 40.0 ± 12.9 years) were included: 20 055 (males: 4182, females: 15 873, mean age: 50.2 ± 13.2 years) were untreated and 7859 (males: 1067, females: 6792, mean age: 46.1 ± 11.5 years) were under L-T_4 treatment for nodular goiter (L-T_4-treated patients).

All patients were submitted to thyroid ultrasound, and all untreated patients with nodules greater than 1 cm were submitted to a technetium-99m-pertechnetate scintiscan. According to clinical findings, ultrasound examination, and thyroid scintiscan, the patients were subdivided into two diagnostic groups. The patients with a solitary nodule (SN) had a single, cold nodule in a normal or slightly enlarged thyroid gland. The multinodular goiter (MNG) patients presented a goiter with multiple nodules during ultrasound examination. During thyroid scan, they only had cold nodules or both cold and 'hot' nodules. In these patients, FNA biopsy was performed only on cold thyroid nodules. In the untreated group, 6567 patients (males: 1468, females: 5099, mean age: 46.3 ± 13.7 years) were included in the SN group and 13 488 patients (males: 2714, females: 10 774, mean age: 52.1 ± 12.6 years) in the MNG group. In the L-T_4-treated group, 2446 patients (males: 345, females: 2101, mean age: 43.5 ± 12.1 years) were included in the SN group and 5413 patients (males: 722, females: 4691, mean age: 47.3 ± 11.0 years) in the MNG group.

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 IRMA (Delphia Pharmacia, Turku, Finland – normal values – 0.4–3.4 μU/ml). TgAbs and TPOAbs were measured by an Automated Immunoassay Assay (AIA) system (AIA-Pack TgAb, and TPOAb, Tosoh, Tokyo, Japan), and are expressed in U/ml. Normal values were <30 U/ml for TgAbs and <10 U/ml for TPOAbs. TRAbs were measured by a radioreceptor assay (TRAK Assay, Brahms, Berlin, Germany).

FNA and cytological 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 was interpreted by an experienced cytologist (G Di Coscio). The adequacy of aspirates was defined according to the guidelines of The Papanicolaou Society of Cytopathology Task Force on Standards of Practice (1996), and the cytological results were classified according to the criteria of the British Thyroid Association (2007).

Statistical analysis

Free thyroid hormones and TSH values are expressed as median and interquartile range (25–75p). Nonparametric tests (χ^2 or Mann–Whitney) were used wherever appropriate and were considered statistically significant when $P<0.05$.

Results

Free thyroid hormones in untreated and L-T_4-treated patients

Levels of FT_4 and FT_3 in untreated and L-T_4-treated patients are presented in Table 1. As expected, FT_4 was significantly lower (Mann–Whitney $P<0.0001$) in untreated patients (median 10.4 pg/ml, 25–75p 9.2–11.9 pg/ml) than in L-T_4-treated patients (median 12.2 pg/ml, 25–75p 10.5–14.1 pg/ml). Surprisingly, FT_3 levels were slightly, but significantly higher (Mann–Whitney $P<0.0001$) in untreated patients (median 3.8 pg/ml, 25–75p 3.4–4.2 pg/ml) than in L-T_4-treated patients (median 3.7 pg/ml, 25–75p 3.3–4.1 pg/ml).

Table 1 Free thyroid hormone levels (expressed as median and interquartile range) in untreated and L-thyroxine (T_4)-treated patients

	Untreated	L-T_4-treated	*P* value[a]
FT_4 (pg/ml)[a]	10.4 (9.2–11.9)	12.2 (10.5–14.1)	<0.0001
FT_3 (pg/ml)[a]	3.8 (3.4–4.2)	3.7 (3.3–4.1)	<0.0001

[a]Mann–Whitney test.

Serum TSH and frequency of PTC in untreated and L-T_4-treated patients

As expected TSH was significantly higher in untreated patients (median 0.70 μU/ml, 25–75p 0.38–1.14 μU/ml) than in L-T_4-treated patients (median 0.30 μU/ml, 25–75p 0.08–0.62 μU/ml; $P<0.0001$) (Fig. 1 A).

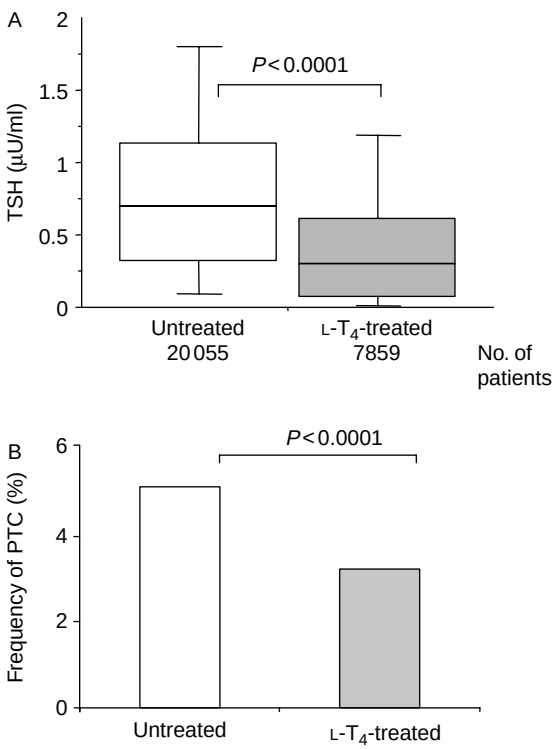

Figure 1 Panel A: box–whiskers plot of TSH levels (μU/ml) in untreated (white columns) and L-T_4-treated (gray columns) patients. Results are reported as median values (black lines), interquartile (25–75th percentiles) range (boxes), and 10–90th percentiles (whiskers); the statistical difference between groups was evaluated using the Mann–Whitney test. TSH was significantly higher in untreated patients than in L-T_4-treated patients (median, interquartile range 0.70 μU/ml, 0.38–1.14 μU/ml versus 0.30 μU/ml, 0.08–0.62 μU/ml; *P* value <0.0001). Panel B: frequency of PTC in untreated (white columns) and L-T_4-treated (gray columns) patients. The overall risk of PTC was significantly higher in untreated patients than in L-T_4-treated patients (5.1 vs 3.2%; χ^2 *P* value <0.0001).

Of the 27 914 patients included in the study, 26 639 had one or more cytologically benign thyroid nodules and 1275 had at least one nodule with a cytology suggestive or indicative of PTC. The overall frequency of PTC (Fig. 1B) was significantly higher in untreated patients than in L-T_4-treated patients (1025/20 055, 5.1 vs 250/7859, 3.2%; χ^2 P value <0.0001). The frequency of PTC in relationship to serum TSH, the odds ratio, and 95% confidence interval is reported in Table 2. Of 27 914 patients, 10 059 (36.0%) had serum TSH below the lower limit of normal range (<0.4 μU/ml) and 127 (0.5%) had serum TSH above the upper limit of normal range (>3.4 μU/ml). Of 27 914 patients, 17 728 (63.5%) had serum TSH within the normal range (≥ 0.4 μU/ml and ≤ 3.4 μU/ml, median 0.9 μU/ml), and they were subdivided into four quartiles. The frequency of PTC was closely related to serum TSH, with it being lowest in patients with TSH levels <0.4 μU/ml (189/10 059, 1.9%) and highest in patients with TSH levels >3.4 μU/ml (21/127, 16.5%), also showing a progressive increase from the lower to the upper quartile of normal range.

The frequency distribution of untreated and L-T_4-treated patients according to their serum TSH is shown in Fig. 2. A significantly higher proportion of L-T_4-treated patients (4560/7859, 58.0%) with respect to untreated patients (5499/20 055, 27.4%; χ^2 P value <0.0001) had serum TSH below the normal range (<0.4 μU/ml). On the contrary, starting from the second quartile of normal TSH values, a significantly higher proportion of untreated patients with respect to L-T_4-treated patients were included in each TSH interval. Overall, a significantly higher proportion of L-T_4-treated patients (6650/7859, 84.6%) with respect to untreated patients (12 599/20 055, 62.8%; χ^2 P value <0.0001) had serum TSH below the median (Fig. 2 inset) in the range of TSH associated with a lower risk of PTC.

Table 2 Frequency, odds ratio (OR), and 95% confidence interval (CI) of papillary thyroid cancer according to TSH levels

TSH (μU/ml)	Frequency of PTC (%)	OR	95% CI	P value
<0.4	189/10 059 (1.9%)	–	–	–
0.40–0.59	137/4134 (3.3%)	1.79	1.42–2.23	0.0005
0.60–0.89	250/5047 (5.0%)	2.72	2.24–3.30	<0.0001
0.90–1.30	296/4406 (6.7%)	3.76	3.12–4.53	<0.0001
1.31–3.40	382/4132 (9.2%)	5.32	4.45–6.36	<0.0001
>3.40	21/127 (16.5%)	10.36	6.34–16.89	<0.0001

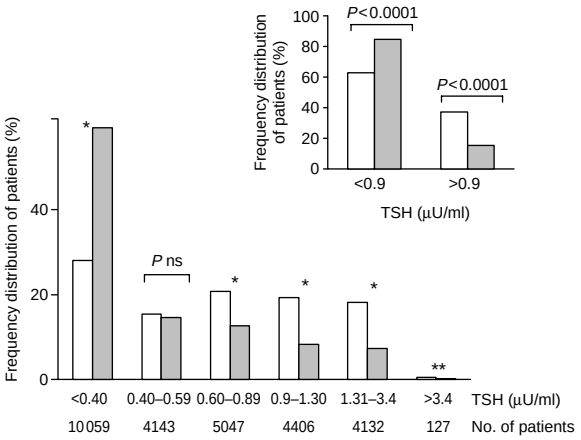

Figure 2 Frequency distribution of untreated (white columns) and L-T_4-treated (gray columns) patients according to their serum TSH. Number of patients in each TSH intervals is also reported. A significantly higher proportion of L-T_4-treated patients (58.0%) with respect to untreated patients (27.4%; χ^2 P value <0.0001) had serum TSH below the normal range (<0.4 μU/ml). On the contrary, starting from the second quartile of normal TSH values, a significantly higher proportion of untreated patients with respect to L-T_4-treated patients were included in each TSH interval (*$P<0.001$). Also, in the small group of patients with TSH levels >3.4 μU/ml, a significantly higher proportion of untreated patients with respect to L-T_4-treated patients were included (**$P=0.004$). The overall frequency distribution of L-T_4-treated and untreated patients below and above the TSH median value of the whole study group (0.9 μU/ml) is reported in the inset. A significantly higher proportion of L-T_4-treated patients with respect to untreated patients had serum TSH below the median (84.6 vs 62.8%; χ^2 P value <0.0001).

Frequency of PTC in untreated and L-T_4-treated patients according to age and serum TSH

The age distribution in untreated and L-T_4-treated patients is reported in Fig. 3A. Untreated patients were significantly older (median age 49 years, 25–75p 40–58 years) than patients under L-T_4 treatment (median age 42 years, 25–75p 33–53 years, $P<0.0001$). In order to rule out the possibility that the different PTC frequency observed in untreated and L-T_4-treated patients could be related to this age difference, we analyzed the risk of PTC according to age, TSH levels, and L-T_4 treatment (Fig. 3B). In all age groups, the frequency of PTC was significantly higher in untreated patients with TSH levels ≥ 0.4 μU/ml than in patients with TSH levels <0.4 μU/ml both untreated and under L-T_4 treatment. With the exception of the age group 31–40 years, no significant difference of PTC frequency was observed between untreated and L-T_4-treated patients with TSH levels <0.4 μU/ml.

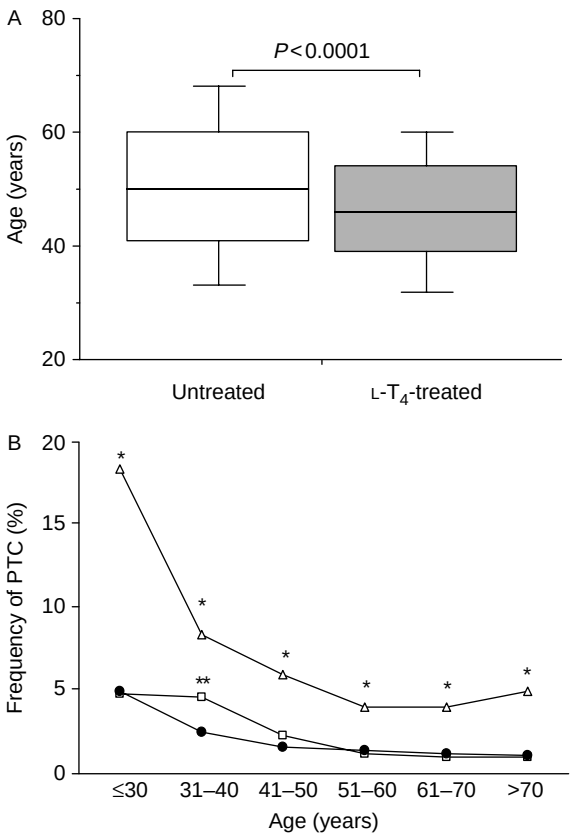

Figure 3 Risk of PTC according to TSH levels, age, and L-T_4 treatment. Panel A: untreated patients (white columns) were significantly older than patients under L-thyroxine treatment (gray columns). Panel B: in all age groups, the frequency of PTC was significantly higher (*$P<0.001$) in untreated patients with TSH levels ≥ 0.4 μU/ml (indicated with the Δ symbol) than in patients with TSH levels <0.4 μU/ml both untreated (indicated with the □ symbol) and under L-thyroxine treatment (indicated with the ● symbol). With the exception of patients aged 31–40 years (**$P=0.02$), no significant age-dependent difference of PTC frequency was observed between untreated and L-T_4-treated patients with TSH levels <0.4 μU/ml.

Frequency of PTC in untreated and L-T_4-treated patients according to clinical diagnosis and serum TSH

In 18 251/18 901 (96.6%) MNG patients, all thyroid nodules were cytologically benign, and in 650/18 901 (3.4%) patients, at least one nodule had a cytology suggestive or indicative of PTC. Of 9013 SN patients, 8388 (93.1%) had a benign cytology and 625/9013 (6.9%) had a cytology suggestive or indicative of PTC. Thus, the overall prevalence of PTC (Fig. 4 and panel A) was significantly lower in MNG than in SN (3.4 vs 6.9%; χ^2 P value <0.0001). The frequency of PTC remained significantly lower in MNG than in SN both in patients with TSH levels ≥ 0.4 μU/ml (539/11 136, 4.8 vs 544/6617, 8.2%; χ^2 P value <0.0001) and in those with TSH levels <0.4 μU/ml (111/7765, 1.4 vs 81/2396, 3.3%; χ^2 P value <0.0001). The frequency of PTC was always significantly lower in patients with TSH levels <0.4 μU/ml than in those with TSH levels ≥ 0.4 μU/ml both in MNG and in SN.

In patients with TSH levels <0.4 μU/ml (Fig. 4 and panel B), no significant difference was observed between untreated and L-T_4-treated patients both in MNG (65/4453, 1.5 vs 46/3312, 1.4%; χ^2 P value NS) and in SN (41/1113, 3.7 vs 40/1283, 3.1%; χ^2 P value NS).

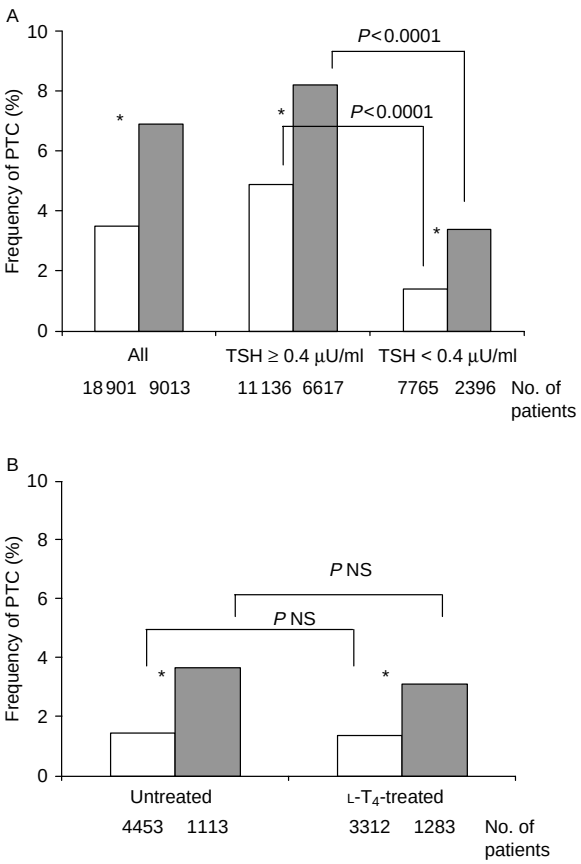

Figure 4 Frequency of PTC according to TSH level, type of goiter (MNG: white columns and SN: gray columns), and L-T_4 treatment. Panel A: the overall risk of PTC was significantly lower (*=χ^2 P value <0.0001) in MNG than in SN (3.5 vs 6.9%). The frequency of PTC remained significantly lower in MNG than in SN both in patients with TSH levels ≥ 0.4 μU/ml (4.9 vs 8.2%) and in those with TSH levels <0.4 μU/ml (1.4 vs 3.3%). Furthermore, the frequency of PTC was always significantly lower in patients with TSH levels <0.4 μU/ml than in those with TSH levels ≥ 0.4 μU/ml both in MNG and in SN. Panel B: in patients with TSH levels <0.4 μU/ml, no significant difference was observed between untreated and L-T_4-treated patients both in MNG and in SN.

Discussion

It has been reported that in patients with nodular thyroid diseases, the risk of thyroid malignancy increases with serum TSH concentrations, and that even within normal ranges, higher TSH values are associated with a higher frequency and more advanced stage of thyroid cancer (Boelaert *et al*. 2006; Haymart *et al*. 2008*b*; Jonklaas *et al*. 2008; Polyzos *et al*. 2008). In a previous study, we have shown that in patients with nodular goiter, the development of thyroid autonomy is associated with a lower risk of PTC, suggesting that thyroid autonomy by reducing TSH levels may slow down cancer progression (Fiore *et al*. 2009). In the present study, we have analyzed the risk of PTC in patients with nodular thyroid disease and low TSH levels due to the spontaneous development of thyroid autonomy or the treatment with L-T_4. We performed a cross-sectional study evaluating the frequency of PTC in a large series of patients with nodular goiter at the time of their first observation in our Department, and included in this study both untreated and L-T_4-treated patients when they were submitted to FNA cytology of thyroid nodules. We focused on PTC as it is the most frequent thyroid cancer, accounting for more than 80% of all thyroid malignancies and the cytological diagnosis of this cancer is usually highly dependable. Furthermore, in a previous study, we validated the reliability of cytological examination in patients submitted to surgery, showing very high positive and negative predictive values of cytology, 99.5 and 98.3% respectively (Fiore *et al*. 2009).

In the study group, as expected, TSH was significantly higher and FT_4 was significantly lower in untreated patients than in L-T_4-treated patients. Surprisingly, FT_3 levels were slightly, but significantly higher in untreated patients than in L-T_4-treated patients. The low TSH level induced by L-T_4 treatment and the subsequent reduction of secretion of T_3 from the thyroid could explain the lower FT_3 levels observed in L-T_4-treated patients. Furthermore, the untreated group included patients with nodular goiter and thyroid autonomy, which may contribute to the higher FT_3 levels detected in this group. We have analyzed the frequency of PTC according to the serum TSH of the patient. A significant proportion of patients (36.0%) had serum TSH below the lower limit of normal range (<0.4 μU/ml) due to spontaneous functional autonomy or to the treatment with L-T_4. Few patients (0.5%) had serum TSH above the upper limit of normal range (>3.4 μU/ml). The latter patients had undetectable thyroid autoantibodies and no signs of thyroiditis during thyroid ultrasound and may be considered as 'euthyroid outliers' of this study group (Andersen *et al*. 2003) or subjects with mild hypothyroidism with no signs of thyroid autoimmunity. The frequency of PTC was closely related to serum TSH, with it being lowest in patients with TSH levels <0.4 μU/ml (1.9%) and highest in patients with TSH levels >3.4 μU/ml (16.5%) and also showing a progressive increase from the lower to the upper quartile of normal range of TSH. This relationship between higher TSH values and increased risk of PTC has already been reported (Boelaert *et al*. 2006, Haymart *et al*. 2008*b*, Jonklaas *et al*. 2008, Polyzos *et al*. 2008, Fiore *et al*. 2009). In the present study, we have analyzed a large series of patients with nodular goiter and focused on the treatment with L-T_4. The overall frequency of PTC was significantly lower in L-T_4-treated patients (3.2%) than in untreated patients (5.1%) due to the different frequency distribution of these patients according to their TSH levels. Actually, a significantly higher proportion of L-T_4-treated patients with respect to untreated patients had serum TSH below the median (0.90 μU/ml), with it being included in the range of TSH associated with a lower risk of PTC.

L-T_4 is a common medical treatment for nodular goiter, and while its real effectiveness is still debated, it is widely agreed that L-T_4-induced suppression of TSH secretion shrinks thyroid nodules by preventing the growth-promoting effect of TSH on thyroid cells (Hegedus *et al*. 2003, Filetti *et al*. 2006). Furthermore, 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), while no data are available on a possible protective effect on thyroid cancer development. Experimental animal models have shown an increased development of thyroid carcinomas in mice (Schaller & Stevenson 1966) and golden hamsters (Fortner *et al*. 1960) fed on a low-iodine diet, and it has already been proposed that thyroid overstimulation by TSH leads to hyperplasia and eventually to the development of cancer. TSH suppression in rats exposed to radioiodine was shown to prevent thyroid nodules and thyroid cancers (Goldberg *et al*. 1964). Our results, together with those obtained in patients with thyroid autonomy (Fiore *et al*. 2009), confirm the hypothesis that low TSH levels could slow down cancer progression. PTC frequently presents genetic alterations leading to the activation of the mitogen-activated protein kinase

(MAPK) signaling pathway, mainly point mutations of the *BRAF* gene and RET/PTC rearrangements (Ciampi & Nikiforov 2007, Nikiforova & Nikiforov 2008). It is conceivable that in patients with thyroid autonomy or treated with L-T4, low TSH levels could reduce the probability that mutated oncogenes may cause clinically detectable cancer. However, our data do not allow us to draw any conclusion about the possible role of TSH in the genesis of such mutations, and we believe that specific studies involving animal models are also required to address this point.

We need to underscore that this is a cross-sectional study that included all patients at their first observation in our Department. In this study, it was not possible to establish how long the patients had been treated with L-T_4 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 had been under treatment with L-T_4 for nodular goiter 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-T_4 treatment. On the other hand, all the studies reported in the literature on the relationship between TSH and frequency of PTC are cross-sectional (Boelaert *et al*. 2006, Haymart *et al*. 2008*b*, Jonklaas *et al*. 2008, Polyzos *et al*. 2008, Fiore *et al*. 2009) and do not deal with the problem of TSH level on time. Longitudinal case–control studies are required to address this question.

Untreated and L-T_4-treated patients had different age distribution, with untreated patients being significantly older than patients under L-T_4 treatment. For this reason, we analyzed the risk of PTC according to age and TSH levels. In all age groups, the frequency of PTC was significantly higher in untreated patients with TSH levels ≥ 0.4 μU/ml than in patients with TSH levels < 0.4 μU/ml both untreated and under L-T_4 treatment, suggesting that the protective effect of low TSH levels is not age dependent. No significant difference in the frequency of PTC was observed between untreated and L-T_4-treated patients with TSH levels < 0.4 μU/ml in all age groups with the exception of patients aged 31–40 years. We do not have an explanation for this observation, and can only hypothesize that unknown factors involved in the pathogenesis of PTC may play a role in this age group.

We have also analyzed the relationship between TSH levels and frequency of PTC according to the type of goiter. Our data show that the frequency of PTC is significantly lower in MNG patients than in SN patients. This result confirms the data obtained in previous studies (Christensen *et al*. 1984, Boelaert *et al*. 2006, Fiore *et al*. 2009), while in other studies the same risk of malignancy has been observed in patients with multiple thyroid nodules or SNs (Belfiore *et al*. 1992, Marqusee *et al*. 2000, Papini *et al*. 2002). Furthermore, in a recent study, a higher likelihood of malignancy in SN with respect to nonsolitary thyroid nodule was reported, although the risk of malignancy per patient was the same and was independent of the number of nodules (Frates *et al*. 2006). The different frequency of PTC in patients with MNG or SN reported in the literature may be related to the different selection of patients (histological instead of cytological series). Whatever is the relative risk of PTC in SN versus MNG, in the present study it is clear that in both clinical settings the frequency of PTC is significantly higher in patients with TSH levels ≥ 0.4 μU/ml than in those with TSH levels < 0.4 μU/ml, and in the latter group, no significant difference is observed between untreated and L-T_4-treated patients. These data indicate that low levels of TSH are associated with a lower frequency of clinically detectable PTC, independently from the type of goiter.

On the whole, our data indicate that a reduced risk of PTC is present in patients with nodular goiter and low TSH levels due to both the spontaneous development of thyroid autonomy and the treatment with L-T_4. However, these data are not sufficient to advise L-T_4 treatment in all patients with nodular goiter with the aim of reducing the risk of PTC. First, it is possible that a large part of the cancers we have detected would not have had deleterious effects if not removed by surgery, as longitudinal studies on this matter are missing. Secondly, and more importantly, L-T_4 treatment is not without risk, with the negative effects of TSH-suppressive treatment with L-T_4 on cardiovascular system in aged subjects (Biondi *et al*. 2002, Fazio *et al*. 2004) and development of osteoporosis in post-menopausal women (Williams 1997, Heijckmann *et al*. 2005) being well documented. Longitudinal case–control studies and careful evaluation of the cost-effectiveness of L-T_4 treatment are required to address this question.

In conclusion, our results confirm a direct relationship between TSH levels and risk of PTC, and show that treatment with L-T_4, reducing serum TSH, decreases the risk of PTC in patients with nodular goiter independently of age and type of nodularity.

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.

Funding

This work was supported by the Italian 'Ministero dell'Università e della Ricerca Scientifica' (MURST) research program (grant number 2007 RJ7AP8): protein, metabolomic, fingerprint and gene expression profile of thyroid nodules with follicular proliferation cytology: identification of new marker to distinguish benign and malignant thyroid nodules.

References

Andersen S, Bruun NH, Pedersen KM & Laurberg P 2003 Biologic variation is important for interpretation of thyroid function tests. *Thyroid* **13** 1069–1078.

Belfiore A, La Rosa GL, La Porta GA, Giuffrida D, Milazzo G, Lupo L, Regalbuto C & Vigneri R 1992 Cancer risk in patients with cold thyroid nodules: relevance of iodine intake, sex, age, and multinodularity. *American Journal of Medicine* **93** 363–369.

Biondi B, Palmieri EA, Lombardi G & Fazio S 2002 Effects of subclinical thyroid dysfunction on the heart. *Annals of Internal Medicine* **137** 904–914.

Biondi B, Filetti S & Schlumberger M 2005 Thyroid-hormone therapy and thyroid cancer: a reassessment. *Nature Clinical Practice. Endocrinology & Metabolism* **1** 32–40.

Boelaert K, Horacek J, Holder RL, Watkinson JC, Sheppard MC & Franklyn JA 2006 Serum thyrotropin concentration as a novel predictor of malignancy in thyroid nodules investigated by fine-needle aspiration. *Journal of Clinical Endocrinology and Metabolism* **91** 4295–4301.

British Thyroid Association 2007 Fine-needle aspiration cytology. In Report of the Thyroid Cancer Guidelines Update Group, edn 2, pp 9–10. London: Royal College of Physicians.

Christensen SB, Ljungberg O & Tibblin S 1984 Thyroid carcinoma in Malmo, 1960–1977. Epidemiologic, clinical, and prognostic findings in a defined urban population. *Cancer* **53** 1625–1633.

Ciampi R & Nikiforov YE 2007 RET/PTC rearrangements and BRAF mutations in thyroid tumorigenesis. *Endocrinology* **148** 936–941.

Farid NR, Shi Y & Zou M 1994 Molecular basis of thyroid cancer. *Endocrine Reviews* **15** 202–232.

Fazio S, Palmieri EA, Lombardi G & Biondi B 2004 Effects of thyroid hormone on the cardiovascular system. *Recent Progress in Hormone Research* **59** 31–50.

Feldt-Rasmussen U 2001 Iodine and cancer. *Thyroid* **11** 483–486.

Filetti S, Durante C & Torlontano M 2006 Nonsurgical approaches to the management of thyroid nodules. *Nature Clinical Practice. Endocrinology & Metabolism* **2** 384–394.

Fiore E, Rago T, Provenzale A, Scutari M, Ugolini C, Basolo F, Di Coscio G, Berti P, Grasso L, Elisei R *et al.* 2009 Lower levels of TSH are associated to a lower risk of papillary thyroid cancer in patients with thyroid nodular disease: thyroid autonomy may play a protective role. *Endocrine Related Cancer* **16** 1251–1260.

Fortner JG, George PA & Sternberg SS 1960 Induced and spontaneous thyroid cancer in the Syrian (golden) hamster. *Endocrinology* **6** 364–376.

Frates MC, Benson CB, Doubilet PM, Kunreuther E, Contreras M, Cibas ES, Orcutt J, Moore FD Jr, Larsen PR, Marqusee E *et al.* 2006 Prevalence and distribution of carcinoma in patients with solitary and multiple thyroid nodules on sonography. *Journal of Clinical Endocrinology and Metabolism* **91** 3411–3417.

Goldberg RC, Lindsay S, Nichols CW Jr & Chaikoff IL 1964 Induction of neoplasms in thyroid glands of rats by subtotal thyroidectomy and by the injection of one microcurie of i-131. *Cancer Research* **24** 35–43.

Haymart MR, Glinberg SL, Liu J, Sippel RS, Jaume JC & Chen H 2008*a* Higher serum TSH in thyroid cancer patients occurs independent of age and correlates with extrathyroidal extension. *Clinical Endocrinology*.

Haymart MR, Repplinger DJ, Leverson GE, Elson DF, Sippel RS, Jaume JC & Chen H 2008*b* Higher serum thyroid stimulating hormone level in thyroid nodule patients is associated with greater risks of differentiated thyroid cancer and advanced tumor stage. *Journal of Clinical Endocrinology and Metabolism* **93** 809–814.

Hegedus L, Bonnema SJ & Bennedbaek FN 2003 Management of simple nodular goiter: current status and future perspectives. *Endocrine Reviews* **24** 102–132.

Heijckmann AC, Huijberts MS, Geusens P, de Vries J, Menheere PP & Wolffenbuttel BH 2005 Hip bone mineral density, bone turnover and risk of fracture in patients on long-term suppressive L-thyroxine therapy for differentiated thyroid carcinoma. *European Journal of Endocrinology* **153** 23–29.

Jonklaas J, Nsouli-Maktabi H & Soldin SJ 2008 Endogenous thyrotropin and triiodothyronine concentrations in individuals with thyroid cancer. *Thyroid* **18** 943–952.

Marqusee E, Benson CB, Frates MC, Doubilet PM, Larsen PR, Cibas ES & Mandel SJ 2000 Usefulness of ultrasonography in the management of nodular thyroid disease. *Annals of Internal Medicine* **133** 696–700.

Mazzaferri EL 1991 Treating differentiated thyroid carcinoma: where do we draw the line? *Mayo Clinic Proceedings* **66** 105–111.

Mazzaferri EL 1999 An overview of the management of papillary and follicular thyroid carcinoma. *Thyroid* **9** 421–427.

Mazzaferri EL & Jhiang SM 1994 Long-term impact of initial surgical and medical therapy on papillary and follicular thyroid cancer. *American Journal of Medicine* **97** 418–428.

Mazzaferri EL & Young RL 1981 Papillary thyroid carcinoma: a 10 year follow-up report of the impact of therapy in 576 patients. *American Journal of Medicine* **70** 511–518.

Nikiforova MN & Nikiforov YE 2008 Molecular genetics of thyroid cancer: implications for diagnosis, treatment and prognosis. *Expert Review of Molecular Diagnostics* **8** 83–95.

Papini E, Petrucci L, Guglielmi R, Panunzi C, Rinaldi R, Bacci V, Crescenzi A, Nardi F, Fabbrini R & Pacella CM 1998 Long-term changes in nodular goiter: a 5-year prospective randomized trial of levothyroxine suppressive therapy for benign cold thyroid nodules. *Journal of Clinical Endocrinology and Metabolism* **83** 780–783.

Papini E, Guglielmi R, Bianchini A, Crescenzi A, Taccogna S, Nardi F, Panunzi C, Rinaldi R, Toscano V & Pacella CM 2002 Risk of malignancy in nonpalpable thyroid nodules: predictive value of ultrasound and color-Doppler features. *Journal of Clinical Endocrinology and Metabolism* **87** 1941–1946.

Polyzos SA, Kappaita M, Efstathiadou Z, Poulakos P, Slavakis A, Sofianou D, Flaris N, Leontsini M, Kourtis A & Avramidis S 2008 Serum thyrotropin concentration as a biochemical predictor of thyroid malignancy in patients presenting with thyroid nodules. *Journal of Cancer Research and Clinical Oncology* **134** 953–960.

Schaller RT Jr & Stevenson JK 1966 Development of carcinoma of the thyroid in iodine-deficient mice. *Cancer* **19** 1063–1080.

Sipos JA & Mazzaferri EL 2008 The therapeutic management of differentiated thyroid cancer. *Expert Opinion on Pharmacotherapy* **9** 2627–2637.

The Papanicolaou Society of Cytopathology Task Force on Standards of Practice 1996 Guidelines of the Papanicolaou Society of Cytopathology for the examination of fine-needle aspiration specimens from thyroid nodules. *Diagnostic Cytopathology* **15** 84–89.

Williams JB 1997 Adverse effects of thyroid hormones. *Drugs and Aging* **11** 460–469.

Wynford-Thomas D 1993 Molecular genetics of thyroid cancer. *Trends in Endocrinology and Metabolism* **4** 224–232.