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

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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-T₄) 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-T₄ ($n=7859$). L-T₄-treated patients with respect to untreated patients presented significantly lower serum TSH (median, interquartile range: $0.30\ \mu\text{U/ml}$, $0.08\text{--}0.62\ \mu\text{U/ml}$ versus $0.70\ \mu\text{U/ml}$, $0.38\text{--}1.14\ \mu\text{U/ml}$; $P<0.0001$) 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 ($<0.4\ \mu\text{U/ml}$; $189/10\,059$, 1.9%) and highest in patients with TSH above the normal range ($>3.4\ \mu\text{U/ml}$; $21/127$, 16.5%), also showing a progressive increase from the lower to the upper quartile of normal range. A significantly higher proportion of L-T₄-treated patients ($6650/7859$, 84.6%) had serum TSH below the median ($0.90\ \mu\text{U/ml}$) 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-T₄ is responsible for the reduction of serum TSH and is associated with a decreased frequency of PTC.

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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-T₄) 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-T₄ treatment is still debated (Hegedus *et al.* 2003, Filetti *et al.* 2006). L-T₄ 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-T₄, presenting a

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. 2008a,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-T₄ treatment on thyroid cancer development.

In this study, our aim was to investigate the possible relationship between L-T₄ 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-T₄ treatment or were treated with L-T₄ 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-T₄ treatment. A significantly higher proportion of L-T₄-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-T₄ 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-T₄ treatment for nodular goiter (L-T₄-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-perchnetate 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-T₄-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 T₄ (FT₄) and triiodothyronine (FT₃) were measured by RIA (FT₄ by Liso-Phase kit – normal values 7–17 pg/ml; FT₃ 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-T₄-treated patients

Levels of FT₄ and FT₃ in untreated and L-T₄-treated patients are presented in Table 1. As expected, FT₄ 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-T₄-treated patients (median 12.2 pg/ml, 25–75p 10.5–14.1 pg/ml). Surprisingly, FT₃ 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-T₄-treated patients (median 3.7 pg/ml, 25–75p 3.3–4.1 pg/ml).

Serum TSH and frequency of PTC in untreated and L-T₄-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-T₄-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-T₄-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-T₄-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-T₄-treated (gray columns) patients. The overall risk of PTC was significantly higher in untreated patients than in L-T₄-treated patients (5.1 vs 3.2%; χ^2 P value < 0.0001).

Table 1 Free thyroid hormone levels (expressed as median and interquartile range) in untreated and L-thyroxine (T₄)-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

^aMann–Whitney test.

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-T₄-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 μ U/ml) and 127 (0.5%) had serum TSH above the upper limit of normal range (>3.4 μ U/ml). Of 27 914 patients, 17 728 (63.5%) had serum TSH within the normal range ($\geq$ 0.4 μ U/ml and $\leq$ 3.4 μ U/ml, median 0.9 μ U/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 μ U/ml (189/10 059, 1.9%) and highest in patients with TSH levels >3.4 μ U/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-T₄-treated patients according to their serum TSH is shown in Fig. 2. A significantly higher proportion of L-T₄-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 μ U/ml). On the contrary, starting from the second quartile of normal TSH values, a significantly higher proportion of untreated patients with respect to L-T₄-treated patients were included in each TSH interval. Overall, a significantly higher proportion of L-T₄-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 (μ U/ml)	Frequency of PTC (%)	OR	95% CI	<i>P</i> 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-T₄-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-T₄-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 μ U/ml). On the contrary, starting from the second quartile of normal TSH values, a significantly higher proportion of untreated patients with respect to L-T₄-treated patients were included in each TSH interval (**P*<0.001). Also, in the small group of patients with TSH levels >3.4 μ U/ml, a significantly higher proportion of untreated patients with respect to L-T₄-treated patients were included (***P*=0.004). The overall frequency distribution of L-T₄-treated and untreated patients below and above the TSH median value of the whole study group (0.9 μ U/ml) is reported in the inset. A significantly higher proportion of L-T₄-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-T₄-treated patients according to age and serum TSH

The age distribution in untreated and L-T₄-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-T₄ 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-T₄-treated patients could be related to this age difference, we analyzed the risk of PTC according to age, TSH levels, and L-T₄ treatment (Fig. 3B). In all age groups, the frequency of PTC was significantly higher in untreated patients with TSH levels $\geq$ 0.4 μ U/ml than in patients with TSH levels <0.4 μ U/ml both untreated and under L-T₄ treatment. With the exception of the age group 31–40 years, no significant difference of PTC frequency was observed between untreated and L-T₄-treated patients with TSH levels <0.4 μ U/ml.

Figure 3 Risk of PTC according to TSH levels, age, and L-T₄ 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 $\mu\text{U/ml}$ (indicated with the Δ symbol) than in patients with TSH levels < 0.4 $\mu\text{U/ml}$ both untreated (indicated with the $\square$ symbol) and under L-thyroxine treatment (indicated with the $\bullet$ 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-T₄-treated patients with TSH levels < 0.4 $\mu\text{U/ml}$.

Frequency of PTC in untreated and L-T₄-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 $\mu\text{U/ml}$ (539/11 136,

4.8 vs 544/6617, 8.2%; χ^2 P value < 0.0001) and in those with TSH levels < 0.4 $\mu\text{U/ml}$ (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 $\mu\text{U/ml}$ than in those with TSH levels ≥ 0.4 $\mu\text{U/ml}$ both in MNG and in SN.

In patients with TSH levels < 0.4 $\mu\text{U/ml}$ (Fig. 4 and panel B), no significant difference was observed between untreated and L-T₄-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-T₄ treatment. Panel A: the overall risk of PTC was significantly lower (* $=\chi^2$ 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 $\mu\text{U/ml}$ (4.9 vs 8.2%) and in those with TSH levels < 0.4 $\mu\text{U/ml}$ (1.4 vs 3.3%). Furthermore, the frequency of PTC was always significantly lower in patients with TSH levels < 0.4 $\mu\text{U/ml}$ than in those with TSH levels ≥ 0.4 $\mu\text{U/ml}$ both in MNG and in SN. Panel B: in patients with TSH levels < 0.4 $\mu\text{U/ml}$, no significant difference was observed between untreated and L-T₄-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. 2008b; 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-T₄. 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-T₄-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 FT₄ was significantly lower in untreated patients than in L-T₄-treated patients. Surprisingly, FT₃ levels were slightly, but significantly higher in untreated patients than in L-T₄-treated patients. The low TSH level induced by L-T₄ treatment and the subsequent reduction of secretion of T₃ from the thyroid could explain the lower FT₃ levels observed in L-T₄-treated patients. Furthermore, the untreated group included patients with nodular goiter and thyroid autonomy, which may contribute to the higher FT₃ 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-T₄. 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. 2008b, 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-T₄. The overall frequency of PTC was significantly lower in L-T₄-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-T₄-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-T₄ is a common medical treatment for nodular goiter, and while its real effectiveness is still debated, it is widely agreed that L-T₄-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-T₄ 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-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 had been under treatment with L-T₄ 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-T₄ 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.* 2008b, 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-T₄-treated patients had different age distribution, with untreated patients being significantly older than patients under L-T₄ 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 μ U/ml than in patients with TSH levels < 0.4 μ U/ml both untreated and under L-T₄ 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-T₄-treated patients with TSH levels < 0.4 μ U/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 μ U/ml than in those with TSH levels < 0.4 μ U/ml, and in the latter group, no significant difference is observed between untreated and L-T₄-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-T₄. However, these data are not sufficient to advise L-T₄ 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-T₄ treatment is not without risk, with the negative effects of TSH-suppressive treatment with L-T₄ 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-T₄ 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-T₄, 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.

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