An elevated level of TSH might be predictive of differentiated thyroid cancer

Le niveau de TSH, facteur prédictif de cancer différencié de la thyroïde ?

Anne Dorange a, Stéphane Triau e, Stéphanie Mucci-Hennekinne f, Alain Bizon g, Sandrine Laboureaux-Soares a, Frédéric Illouz a,b, Patrice Rodien a,b,c,d, Vincent Rohmer a,* b c d

a Département d’endocrinologie diabétologie nutrition, CHU d’Angers, 4, rue Larrey, 49933 Angers cedex 9, France
b Centre de référence des pathologies de la réceptivité hormonale, CHU d’Angers, 4, rue Larrey, 49933 Angers cedex 9, France
c Inserm U694, 4, rue Larrey, 49933 Angers cedex 9, France
d Université d’Angers, 40, rue de Rennes, BP 73532, 49035 Angers cedex, France
e Département d’anatomopathologie, CHU d’Angers, 4, rue Larrey, 49933 Angers cedex 9, France
f Département de chirurgie viscérale, CHU d’Angers, 4, rue Larrey, 49933 Angers cedex 9, France
g Département d’oto-rhino-laryngologie, CHU d’Angers, 4, rue Larrey, 49933 Angers cedex 9, France

Available online 23 November 2011

Résumé

La thérapie frénatrice de la thyroestimuline (TSH) par les hormones thyroïdiennes améliore la survie de sujets opérés d’un cancer différencié de la thyroïde. Le niveau de TSH serait différent selon le type de nodule. L’objectif de cette étude était de comparer de manière rétrospective le niveau de TSH entre deux groupes de sujets ayant bénéficié d’une thyroïdectomie totale pour un nodule, appariés sur le sexe, l’âge et l’état d’endocrinologie nutrition. Une différence significative existait entre les deux groupes en termes d’âge, de sexe, d’antécédents familiaux de maladie thyroïdiennes ou d’auto-immunité thyroïdienne. Les patients dont l’histologie définitive était maligne avaient un niveau moyen de TSH significativement plus élevé que les patients porteurs d’un maladie bénigne (1,55 mU/l versus 0,96 mU/l, p = 0,003). Le risque de cancer était plus important quand la TSH était dans le tertile supérieur de la norme. Il n’y avait pas de corrélation entre le risque de cancer thyroïdien et l’âge, le sexe, les antécédents familiaux de maladie thyroïdienne ou le statut ménopausique. Le risque relatif d’avoir un carcinome thyroïdien était plus important quand les contours nodulaires étaient flous ou en présence de microcalcifications centrales. Ces données confirment une tendance à des valeurs de base de TSH plus élevées chez des sujets présentant un cancer différencié de la thyroïde. Cependant, il semble difficile de définir un seuil qui permettrait de savoir en préopératoire si le nodule est bénin ou malin.
© 2011 Elsevier Masson SAS. Tous droits réservés.

Abstract

Suppression therapy of thyreostimulin (TSH) using thyroid hormones improves survival of subjects operated for differentiated thyroid cancer. The TSH level might be different depending on the type of nodule. The objective of this study was to compare retrospectively the TSH level between two groups of subjects who underwent total thyroidectomy for a nodule, matched on sex, ethnicity, age and biological method of TSH measurement, one whose final histology was benign and one malignant. There was no significant difference between the two groups in terms of age, sex, family history of thyroid disease or thyroid autoimmunity. The subjects, whose final histology was malignant, had a mean TSH level significantly higher than subjects with benign disease (1.55 mU/l versus 0.96 mU/l, \( P = 0.003 \)). Cancer risk was greater when the TSH was in the upper tertile of normal range. There was no correlation between the risk of thyroid cancer and age, sex, family history of thyroid disease, or menopausal status. The relative risk of having thyroid carcinoma was higher when the margins of nodules were blurring or in the presence of microcalcifications. These data confirm a trend toward baseline values of TSH higher in subjects with a thyroid-differentiated cancer. However, we could not define a preoperative threshold that would reliably determine the malignant or benign nature of the nodule.
© 2011 Elsevier Masson SAS. All rights reserved.

1. Introduction

Thyroid nodules are frequent with estimated prevalence and incidence in the adult population of 4–7% and 100 per
100,000 respectively. Non-medullary differentiated thyroid cancer (DTC) is relatively rare, but its incidence has been increasing for the last 30 years. In France, 3500 new cases are diagnosed each year [1]. This increase is probably due to improvements in ultrasonographic techniques, to the discovery of smaller cancers on final histological examination and also to the possible exposure to environmental endocrine disrupters, whose impact is still under-evaluated. Yet, DTC is a good prognosis cancer. It disseminates only in 10% of cases, essentially to the bone and/or the lung. Node recurrence concerns only 7% of cases. The 10-year survival rate is about 93% for papillary variants and 85% for follicular variants [2].

The main objective in the management of thyroid nodules is to differentiate cancers from benign neoplasms before surgery. Several clinic criteria were proposed such as male gender, age less than 20 or greater than 60 years, hard consistency, irregular shape, previous children irradiation, or some familial history such as Gardner’s syndrome [3–6]. Some ultrasonographic features are also suggestive of malignancy, such as the absence of hypoechoic halo surrounding the lesion, irregular margins, central microcalcifications or marked intra-nodular blood flow [7,8]. However, the specificity and sensitivity of these parameters are not sufficient to make a decision. Similarly, the gold-standard fine-needle cytology (FNC) sometimes fails and needs to be repeated [9]. So, currently, surgical excision is recommended for all malignant cytologies and also advocated for suspicious cytologies. Thus, surgery, which can be associated with significant comorbidities (hypothyroidism or recurrent nerve lesions), is sometimes performed in benign diseases instead of simple monitoring and inversely, cancer management can be delayed. Therefore, identifying new risk factors of DTC would be useful to improve therapeutic strategies in targeted populations.

DTC cells express TSH receptors (TSH-R) at their surface and TSH could be implicated in thyroid oncogenesis. This hypothesis is supported by the fact that the suppression of this signal by exogenous thyroid hormones, exerting a negative feedback on the thyreotrop axis, improves the survival rate of patients with DTC, after a combined curative treatment of surgery and radioidine [10].

Recent studies showed that TSH could be a risk factor of malignancy, when its level is at the upper limit or above the normal range [11–13]. These studies have nonetheless some bias, including the inclusion of papillary microcarcinomas, the absence of histological confirmation of the diagnosis, and the inclusion of subgroups with TSH outside the normal range. The main purpose of this study was to confirm the predictive value of TSH in patients who underwent total thyroidectomy for a thyroid nodule, by comparing DTC patients with adenoma subjects, matched for age and sex.

2. Material and methods

We retrospectively reviewed the records of French patients, aged from 17 to 71 years, who underwent thyroid surgery for a nodule at the Oto-rhino-laryngology and the Visceral Surgery Departments of the University Hospital of Angers between January 1987 and March 2009. About 300 thyroidectomies for nodule are performed each year in these departments. All the subjects were followed in the Endocrine Department of the same hospital.

All subjects were selected from a list established through anamnepathological diagnostic coding software, used by the medical group of Angers. The main code was thyroidectomy for a nodular lesion. The nodule could be solitary or within multinodular goiter (MNG). First, we analysed the cases of cancer at the final histological examination. In this group, the size of the main lesion was to be larger than 10 mm. Then, we matched this group of cancer cases (DTC) with cases presenting a benign disease (BD), based on age, ethnicity and sex. All patients recruited should have a stable dose of any medication known to affect thyroid hormones metabolism.

Non-inclusion criteria were: absence of data on TSH levels, medullary thyroid carcinoma on the histological examination, subjects presenting with preoperative thyroid dysfunction, such as hypo- or hyperthyroidism, TSH suppressive therapy with Levothyroxine before surgery, corticoid administration longer than 1 month, and any severe chronic disease, such as renal insufficiency, hepatic or cardiac disease. Pregnant or lactating women were excluded.

Data collected were age at diagnosis (date of thyroidectomy realisation), sex, ethnicity, suspicious nodular diameter as determined by the final histological examination, familial history of any thyroid disease (goiter, nodule, thyroid cancer) and menopausal status of women. In multinodular goiter, the largest nodule was considered as the main nodule. Data obtained from the ultrasound examination were collected to assess criterion suggestive of malignancy, and notably hypoechoic characteristics, presence of irregular margins and central microcalcifications. When available, data from Doppler analysis about the type of blood flow were collected. Serum TSH, serum-free T4 (FT4) and thyroid antibodies (peroxidase [TPOab] or thyroglobulin antibodies [Tgab]) were measured. FT4 levels could not be analysed because of the heterogeneity of biological assays used during the study period. Similarly, the presence of thyrotropin receptor antibody (TRAK) was not analyzed due to insufficient number of data.

Finally, we recorded the reports of cytology. FNC was performed only in subjects with palpable nodules, to ensure proper location of the needle, since it was realised in our centre without ultrasound guidance. Results were distributed into four categories: benign lesions including adenomatous or colloid nodule including cystic changes and Hashimoto’s thyroiditis; suspicious lesions which included indeterminate lesions (follicular neoplasms, oxyphilic tumors) and lesions with suggestive but not definitive evidence of malignancy; malignant lesions and inadequate cytologies (insufficient number of cells).

Biological data were collected during one day at the Endocrine Department to determine the nature of the nodule. During the same day, were performed biological samples, ultrasound analysis, scintigraphic analysis, and FNC. Non-fasting blood samples were collected in the morning, centrifuged and separated immediately. Only one biological analysis was done before surgery. It could be done several months or years before the surgery. The concentration of TSH was measured with the
immunoluminometric method (ILMA) using the kit Lumitester TSH by Brahms Diagnostica laboratory (Aktiengesellschaft Berlin, Germany). The reference range was 0.1–4.5 mUI/l. Thyroid antibodies were measured with a high sensitive ELISA method. They were considered as absent if the value was less than 50 UI/l.

Ultrasound data were mostly but not exclusively obtained from the Nuclear Medicine Department, using a camera brand HITACHI EUB 315 (ultrasound probe of 7.5 MHz) from 1992 to 2002, and a camera brand SIEMENS ADARA SLC (ultrasound probe barrette of 7.5 MHz) from 2002 to the end of the study period. Some were performed in a private radiology practice without predefined strategy.

2.1. Statistics

The primary objective of the study was to confirm that subjects with DTC had higher TSH levels than subjects with benign nodules.

Statistical analyses were performed using Epi-info and Excel softwares (Microsoft Corp., Redmond, WA). The threshold of significance was set at 0.05 and all tests were bilateral. The results are expressed as mean ± standard deviation (for quantitative variables) or percentages (for categorical variables). A qualitative and quantitative study of missing data was conducted. The two groups were defined according to the benign or malignant status of the nodule, obtained after the final histological examination of the specimen. Risk factors associated with DTC were determined by univariate analysis. The Student’s t-test and Mann-Whitney test were used to compare continuous variables as appropriate. The Chi² test or Fisher exact test were used for comparison of categorical variables as appropriate. The association between each risk factor and DTC was expressed as Odds Ratio (OR) and its 95%-confidence interval. The association between TSH level and other parameters collected were approached by a simple regression analysis. The comparison between tertiles of TSH was performed by ANOVA. Multivariate analysis by multiple logistic regression was used to estimate the adjusted OR and its confidence interval at 95% for each selected factor (conservative threshold 0.20).

3. Results

3.1. Characteristics of samples

Forty-seven patients with DTC were identified from the hospital database and matched with 47 patients with benign disease. Table 1 summarizes the main characteristics of patients and lesions. Age, sex ratio, menopausal status of women and familial history of thyroid disease did not differ between the two groups. All the patients were Caucasian and euthyroid before surgery. In the DTC group, three patients had no FNC before surgery. Malignant lesions were distributed as follows: papillary: 78.7% (n = 37), follicular: 19.1%, and oxyphilic neoplasm: 1/47. Tumor grading was established according to the patients’ age and the anatomopathological TNM classification published in 2004 [14]. The grade was quoted 1 in 61.7% of cases (n = 29), 2 in 17.0% of cases (n = 8) and 3 in 21.3% of cases (n = 10). No patient had a grade 4 lesion. Among the papillary cancers, 70.3%, 21.6% and 8.1% of lesions were quoted grade 1, 2, and 3, respectively. Among the follicular cancers, 22.2% of lesions were quoted grade 1 and 77.8%, grade 3. The oxyphilic lesion was quoted grade one. Several outbreaks of carcinomas were found in 68.1% of cases on the final histological analysis. The mean diameter of the main lesion was significantly larger in the DTC group, compared to the BD group: 24.17 mm (10.00–70.00 mm) vs 18.68 mm (5.00–50.00 mm), P = 0.03. The mean size of the main lesion increased with tumor grade in the DTC group, with a size of 20.41 mm, 26.63 mm and 33.1 mm when the lesion was graded 1, 2 and 3, respectively. There was also a significant difference in lesion size between papillary and follicular carcinomas (22.43 mm vs 32.33 mm, P = 0.01).

3.2. Thyroid function

Mean TSH levels were significantly higher in the DTC group compared to the BD group: 1.55 mIU/l vs 0.96 mIU/l, P = 0.003 (Fig. 1). Fig. 2 shows the mean TSH values for all
Table 1
Comparison of main characteristics between patients with a Differentiated Thyroid Cancer (DTC) and patients with a Benign Disease (BD).

<table>
<thead>
<tr>
<th>Patients characteristics</th>
<th>DTC ($n = 47$)</th>
<th>BD ($n = 47$)</th>
<th>$P$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, years (median (range))</td>
<td>43.47 (17.00–71.00)</td>
<td>44.51 (19.00–69.00)</td>
<td>0.64</td>
</tr>
<tr>
<td>Sex ratio men/women ($n$)</td>
<td>9/38</td>
<td>9/38</td>
<td>0.60</td>
</tr>
<tr>
<td>Familial history of thyroid disease ($n/%$)</td>
<td>14/29.8</td>
<td>13/27.6</td>
<td>0.50</td>
</tr>
<tr>
<td>Menopausal status (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Premenopausal</td>
<td>73.0</td>
<td>71.1</td>
<td>0.52</td>
</tr>
<tr>
<td>Menopausal</td>
<td>21.6</td>
<td>18.4</td>
<td></td>
</tr>
<tr>
<td>Menopausal under hormone therapy</td>
<td>5.4</td>
<td>10.5</td>
<td></td>
</tr>
<tr>
<td>Ultrasonographic features</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Presence of hypoechogenicity (%)</td>
<td>66.7</td>
<td>75.0</td>
<td>0.25</td>
</tr>
<tr>
<td>Presence of Irregular margins (%)</td>
<td>46.7</td>
<td>15.0</td>
<td>0.002</td>
</tr>
<tr>
<td>Presence of central microcalcifications Mi (%)</td>
<td>57.6</td>
<td>17.5</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Nodular size (mm)</td>
<td>24.17 (10–70)</td>
<td>18.68 (5–50)</td>
<td>0.03</td>
</tr>
<tr>
<td>Mean of TSH level (mIU/l)</td>
<td>1.55 $\pm$ 0.90</td>
<td>0.96 $\pm$ 0.52</td>
<td>0.003</td>
</tr>
<tr>
<td>Median of TSH (mIU/l)</td>
<td>1.3 (0.2–4.4)</td>
<td>0.84 (0.16–2.6)</td>
<td>0.003</td>
</tr>
<tr>
<td>Presence of ATPO (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Results of FNC ($n/%$)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Suspicious</td>
<td>19/43.2</td>
<td>14/35.0</td>
<td></td>
</tr>
<tr>
<td>Malignant</td>
<td>13/29.5</td>
<td>0/0</td>
<td></td>
</tr>
<tr>
<td>Benign</td>
<td>6/13.6</td>
<td>17/42.5</td>
<td></td>
</tr>
<tr>
<td>Indeterminate</td>
<td>6/13.6</td>
<td>9/22.5</td>
<td></td>
</tr>
</tbody>
</table>

94 patients arranged in ascending order and coded by diagnosis. Although the range of TSH values is similar for both diagnoses, patients with thyroid cancer generally have higher TSH values across the entire cohort. In the DTC group, median level of TSH was 1.30 mIU/l, compared to 0.84 mIU/l, in the BD group.

We compared the two groups according to their preoperative level of TSH. We realised the breakdown of TSH values into tertiles (0.1–1.0 mIU/l, 1.0–2.0 mIU/l, 2.0–4.5 mIU/l) (Fig. 3). Most patients (59.6%) with benign disease had TSH values within the lowest tertile, whereas 63.8% of the patients with DTC had TSH values in the second tertile. However, a substantial proportion (38.3%) of BD patients also had TSH values in the second tertile and one BD patient had a TSH value in the highest tertile. Nonetheless, the risk of DTC was higher in tertile 2 compared to tertile 1 (OR = 3.43, CI: 1.37–8.57), and this increase was more pronounced when tertile 3 was compared to tertile 1 (OR = 11.67, CI: 2.21–61.48). The OR was 4.3 (CI: 1.79–10.33) when comparing the sum of the two upper tertiles of TSH (1 < TSH < 4.5 mIU/l) to the lowest tertile (0.1 < TSH < 1 mIU/l) (Table 2). In the DTC group, no significant difference of mean level of TSH was found according to the histological variant of cancer ($P = 0.92$), or the tumor stage ($P = 0.95$). The ROC curve, presented on Fig. 4, determined the best compromise of specificity and sensitivity for a threshold of 1.15 mIU/l. However, this threshold cannot be used for a reliable discrimination between benign and malignant lesions, as its sensitivity and specificity were only 66% and 66% respectively. The negative predictive value of this test was low (66%).

Fig. 3. Distribution of patients within tertiles of serum thyrotropin (TSH) (tertile 1: 0.1–1.0 mIU/l; tertile 2: 1.0–2.0 mIU/l; tertile 3: 2.0–4.5 mIU/l) and histology benign disease (BD) vs differentiated thyroid cancer (DTC).
Table 2
Odds ratio (OR) in favour of having Differentiated Thyroid Cancer (DTC) with serum thyrotropin (TSH) within tertiles 2 (1.0–2.0 mIU/l), 3 (2.0–4.5 mIU/l), or 2–3 (1.0–4.5 mIU/l) compared with tertile 1 (0.1–1.0 mIU/l).

<table>
<thead>
<tr>
<th>Tertile</th>
<th>DTC</th>
<th>BD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tertile 2</td>
<td>25</td>
<td>17</td>
</tr>
<tr>
<td>Tertile 1</td>
<td>12</td>
<td>28</td>
</tr>
<tr>
<td>OR = (25 × 28)/(12 × 17) = 3.43 (CI: 1.37–8.57)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tertile 3</td>
<td>10</td>
<td>2</td>
</tr>
<tr>
<td>Tertile 1</td>
<td>12</td>
<td>28</td>
</tr>
<tr>
<td>OR = (10 × 28)/(12 × 2) = 11.7 (CI: 2.21–61.48)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tertiles 2–3</td>
<td>35</td>
<td>19</td>
</tr>
<tr>
<td>Tertile 1</td>
<td>12</td>
<td>28</td>
</tr>
<tr>
<td>OR = (35 × 28)/(12 × 19) = 4.3 (CI: 1.79–10.33)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

3.3. Uni- and multivariate analyses

We performed uni- and multivariate analyses to identify other criteria of malignancy. There was no correlation between the presence of familial history of thyroid disease, or menopausal status and the risk of DTC (Table 1). Ultrasonographic features were significantly different between both groups with 46.7% of lesions in the DTC group exhibiting blur margins, compared to 15.0% in the BD group (P = 0.002). Similarly, 57.6% of lesions in the DTC group had central microcalcifications, compared to only 17.5% of lesions in the BD group (P < 0.001). Hypoechoic lesions were not different between both groups (Table 1). An elevated titer of ATPO was found in respectively 24.3% and 12.8% of cancer and benign cases, but the difference was not significant (P = 0.16). More women had a coexistent thyroiritis (Table 1). We performed a logistic regression analysis simultaneously based on gender and age in 94 subjects. These parameters did not affect the risk of thyroid cancer, with OR of 0.99 (CI: 0.96–1.02, P = 0.68) and 0.98 (CI: 0.35–2.75, P = 0.97) respectively. Another multivariate analysis including age, familial history of thyroid disease, menopausal status of women, and TSH level (threshold chosen: 1 mIU/l) was performed in 75 subjects. A TSH level above 1 mIU/l was the only independent risk factor of DTC with an OR of 4.28 (CI: 1.59–11.52, P = 0.004). In a multivariate analysis, based on 72 patients for whom all data were available, nodule size greater than 20 mm, TSH level above 1 mIU/l and ultrasound features suggestive of malignancy (including the presence of central microcalcifications and nodular irregular margins) were independent risk factors of DTC, with odds ratios of respectively 1.08, 5.83, 4.93 and 6.77 (P < 0.05). The significance disappeared when using a threshold of TSH of 1.15 mIU/l (OR = 3.37, P = 0.07). A final analysis including ultrasound criteria (shape of the nodule, echogenicity, presence of microcalcifications in clusters), size of the lesion, presence of ATPO and TSH level (threshold: 1 mIU/l) included 57 patients. Significant risk factors for DTC were TSH levels above 1 mIU/l (OR = 17.24, CI: 2.32–128.13, P = 0.054), irregular margins and calcifications within the nodule (OR of 18.94, CI: 2.03–177.12, P = 0.01 and 6.16, CI: 1.22–31.15 respectively, P = 0.03), tumor size (RR = 1.11, CI: 1.03–1.20, P = 0.012). The type of echogenicity and the presence of ATPO were not risk factors of DTC with OR of 0.46 (CI: 0.07–3.05, P = 0.42) and 4.76 (CI: 0.58–38.74, P = 0.15) respectively (Table 3). Finally, Table 4 summarizes sensitivity, specificity, positive and negative predictive values of clinical, biological and ultrasonographic criteria as screening tests for thyroid cancer.

4. Discussion

Our study confirms that subjects with DTC have TSH levels significantly higher than those with benign thyroid diseases, regardless of sex and age. This result is particularly evident for the upper quartile of TSH, with an OR of 11.67. It also confirms the importance of detecting ultrasound features suggestive of malignancy, such as blur margins of the nodules and presence of central microcalcifications.

TSH is suspected to play a major role in oncogenesis of DTC, which has been reported in previous studies. In 2006, Boelaert et al. [11] analysed thyroid cytology or histology in a cohort of 1183 patients with a palpable nodule including 92 carcinomas. He found an 11-fold higher risk of DTC for patients

Table 3
Multivariate analysis: independent risk factors of Differentiated Thyroid Cancer (DTC).

<table>
<thead>
<tr>
<th>Size TSH &gt; 1 mIU/l</th>
<th>Hypo</th>
<th>Irregular margins</th>
<th>Mi+</th>
<th>ATPO+</th>
</tr>
</thead>
<tbody>
<tr>
<td>OR</td>
<td>1.11</td>
<td>17.24</td>
<td>0.46</td>
<td>18.94</td>
</tr>
</tbody>
</table>

*P = 0.01; **P = 0.005; ***P = 0.01; ****P = 0.03; Hypo: hypoechogenicity; Mi+: presence of microcalcifications; ATPO+: positivity of thyroperoxydase antibody.
with hypothyroidism (TSH level above 5.5 mIU/l), compared to patients with a normal level of TSH. The OR for TSH values between 1.8 and 5.5 mIU/l and between 1.0 and 1.7 mIU/l, were respectively, 3.88 (P = 0.006) and 2.72 (P = 0.046). In 2008, Haymart et al. [12] investigated the correlation between TSH levels and risk of DTC in 883 patients who underwent thyroid surgery, and confirmed the previous findings for TSH values within the reference range. The mean level of TSH was higher in malignant lesions (n = 241), compared to benign lesions (n = 602), regardless of age less than 45 years or greater or equal to 45 years (P = 0.046 and P = 0.027, respectively). Risk of malignancy was increased when the TSH level was under or above the normal range. Compared to the risk of malignancy when the TSH was less than 0.06 mIU/l, the risk of having thyroid cancer was 2.5, 3.5 and 4.5 times higher for TSH values of 1.4–2.49 mIU/l, 2.5–4.99 mIU/l and above 5 mIU/l respectively. Recently, Jonklaas et al. [13] reported the retrospective analysis of a prospective cohort of patients who had normal levels of TSH and underwent thyroid surgery for a nodule. One third of these patients had a DTC at the final diagnosis, while 5% rates are usually reported in the literature. Nonetheless, she confirmed a significantly higher TSH mean level in the malignant group compared to the benign group (1.50 mIU/l vs 1.01 mIU/l, P = 0.0017). The relative risk of having a DTC was 8.7 (CI: 2.2–33.7) when TSH was in the three upper quartiles of the normal range, compared to the lowest one. Similarly, T3L was lower in the DTC group. The authors concluded that T3L could be a maker of the cellular dedifferentiation level, because T3L, via its action on the nuclear receptors, is a potent regulator of genetic expression. All these studies have some bias, including the facts that the diagnosis of malignancy was sometimes based only on cytology, some patients had a preoperative thyroid dysfunction, some patients were under suppressive therapy by thyroid hormones, and the incidence of cancer cases in two cohorts was higher than that described in epidemiological studies. All these studies have also included micropapillary cancers, whose natural history is probably different from that of tumors above 10 mm. The causative mechanisms of this difference in TSH levels are unknown. In our BD group, such as the benign group of Jonklaas’ study [13], the mean level of TSH is lower than the mean values obtained in epidemiological investigations of the US population. Thus, Hamilton found a mean TSH value of 1.8 mIU/l in individuals without thyroid dysfunction or abnormality [15], which is closed to the average value observed in our group of patients with DTC. Benign nodules could have a degree of autonomy that explains lower TSH levels. Unfortunately, we did not have the opportunity to make a precise analysis of scintigraphic features of each nodule to answer this question. Besides, thyroid proliferation and differentiation depends on a precise process of regulation, and notably hormonal influence (TSH, estrogen), environmental influence (iodine status) and other molecules secreted in situ (such as Vascular Endothelial Growth Factor [VEGF]). All these molecules can have an impact on genes. Germline or somatic mutations may block specific regulatory pathways (p53) or amplify others (TSH-R). Some gene mutations appear to be strongly associated with risk of DTC, such as the B-raf mutation, found in nearly 50% of cases of sporadic papillary cancers [16]. TSH could be a growth factor involved in thyroid tumorigenesis. Thyroid growth is partly controlled by the interaction of TSH/TSH-R, which allows activation of a GTP-protein and its effector, adenyl cyclase, resulting in increasing intracellular cAMP, which stimulates the growth of thyrocytes [17,18]. Some studies suggested that thyroid cancer cells express more TSH-R on their surface. Chia et al. showed that mRNA levels of TSH-R were higher in cancer than in adenoma [19]. However, Shi et al. showed that mRNA levels of TSH-R were lower in cancer, their decrease being correlated with the disease progression, and therefore with the state of dedifferentiation of thyroid cancer [20]. Hoelting et al. analyzed the effect of TSH on cell lines obtained from patients with papillary and follicular cancers and showed that it was biphasic: at low doses, there was acceleration of proliferation but at high doses, growth was inhibited in the two types of cell lines. This TSH-dependent decrease in growth potential can be explained by the loss of expression or functionality of the TSH-R by the cells [21]. In 2000, a French team sequenced the exons of genes encoding the TSH-R and the Gsα protein from four thyroid tumors to search for possible activating mutations that may be responsible for the cAMP cascade activation. No mutation was found and they finally concluded that activating mutations of the TSH-R or the Gsα protein were not involved in thyroid carcinogenesis [22]. Recent data suggested the possible role of decreased expression of type 1 deiodase as an early marker of thyroid dedifferentiation [23]. Animal data showed that TSH suppression after radioactive iodine exposure could prevent thyroid carcinoma development [24], but no human data suggest a protective effect of TSH suppression [25]. Some studies showed no effect of TSH on the thyrocytes growth [26,27]. Apart from TSH, other mechanisms might

### Table 4

<table>
<thead>
<tr>
<th>TSH (mIU/l)</th>
<th>N</th>
<th>Sensitivity (%)</th>
<th>Specificity (%)</th>
<th>PPV (%)</th>
<th>NPV (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>TSH &gt; 0.8</td>
<td>94</td>
<td>89.0</td>
<td>40.0</td>
<td>60.0</td>
<td>79.0</td>
</tr>
<tr>
<td>TSH &gt; 1</td>
<td>94</td>
<td>74.0</td>
<td>59.0</td>
<td>65.0</td>
<td>70.0</td>
</tr>
<tr>
<td>TSH &gt; 1.5</td>
<td>94</td>
<td>55.0</td>
<td>66.0</td>
<td>66.0</td>
<td>66.0</td>
</tr>
<tr>
<td>TSH &gt; 1.3</td>
<td>85</td>
<td>47.0</td>
<td>77.0</td>
<td>70.0</td>
<td>63.0</td>
</tr>
<tr>
<td>I.M.</td>
<td>73</td>
<td>54.0</td>
<td>85.0</td>
<td>78.0</td>
<td>59.0</td>
</tr>
<tr>
<td>Mi+</td>
<td>46</td>
<td>46.0</td>
<td>83.0</td>
<td>73.0</td>
<td>50.0</td>
</tr>
<tr>
<td>I. M. and Mi+</td>
<td>20</td>
<td>70.0</td>
<td>91.0</td>
<td>70.0</td>
<td>70.0</td>
</tr>
</tbody>
</table>

I.M: irregular margins; Mi+: presence of microcalcifications; PPV: positive predictive value; NPV: negative predictive value.
exert a role in thyroid oncogenesis, most of which are still unknown.

A positive correlation between autoimmune thyroiditis and risk of DTC was recently reported [28]. In our cohort, we could not confirm this hypothesis, probably because of the small size of the sample, but we observed a higher frequency of thyroiditis in the DTC group. Cipolla et al. have shown an incidence of 26.7% of Hashimoto’s thyroiditis (HT) in a cohort of 89 subjects who underwent thyroidectomy for thyroid cancer, while the incidence of autoimmunity in the control population, who underwent the same surgery for MNG was only 8.9% (P < 0.02). On the other hand, among the 47 subjects who had total thyroidectomy for autoimmune thyroiditis, 27.6% of micropapillary carcinomas were incidentally found at the final histological analysis [29]. The mechanism of this relationship is unknown. Chronic inflammation could lead to neoplastic transformation, by the activation of cytokines and growth factors, responsible for damages in surrounding stromal cells. This could enhance genetic damage and inappropriate cell proliferation. One study reported the possible role of the PI3K/Akt pathway activation. The Phosphatidylinositol 3-kinase (PI3K), ubiquitous lipid kinase, appears to play a major role in the balance between apoptosis and cell survival and in the inflammatory response, allowing the activation of cytokines and lymphocyte migration. Increased activation of PI3K has been found in cases of ovarian, colon and thyroid cancer. PI3K phosphorylates Akt, which causes the suppression of pro-apoptotic signals and promotes tumorigenesis. Larson found an increased expression of PI3K, Akt1 and Akt2 in cases of thyroiditis associated with thyroid cancer and a decreased expression of PTEN, the PI3K inhibitor [30]. In cases of autoimmune thyroiditis, Giordano showed that follicular cells express both Fas and Fas ligand, which activate a pathway leading to cellular apoptosis [31]. Because of the histopathological similarities between papillary thyroid cancer (PTC) and HT [32], we can extrapolate the possibility that this mechanism is also involved in the relationship between PTC and HT. Several studies confirmed a better prognosis when PTC and HT were associated. In a retrospective cohort of patients with PTC, Kashima et al. reported a 10-year survival rate without recurrence of about 85% among subjects without autoimmune disease, compared to 95% when PTC and HT were associated [33]. However, another hypothesis may be that a rise in TSH level leads to the formation of neoplasms in subjects with HT.

In our study, the difference in TSH levels between the two groups was not correlated with menopausal status of women or familial history of thyroid disease. Genetic susceptibility seems nevertheless to contribute to the risk of thyroid cancer. Thus, a study of all thyroid cancers diagnosed in Sweden between 1958 and 2002, showed that the relative risk to have a PTC was three to six more important if a first-degree-relative have presented a thyroid cancer, compared to subjects without familial history [34]. The impact of estrogen status on the risk of DTC has never been demonstrated. However, the prevalence of thyroid disease is higher among women [35]. Estrogens could either stimulate the TSH secretion, or directly activate the growth of thyroid cells [36–38].

In our multivariate analysis, tumor size appears to be an independent risk factor of DTC. This is discordant with other studies that did not find any correlation between nodular size and malignant risk [39,40]. The Society of Radiologists in Ultrasound (SRU) recommends performing FNC according to ultrasound criteria suggestive of malignancy rather than nodular size [41]. Our results may be different because eight cases of MNG were included in the benign group, with a main lesion size less than 10 mm (n = 5) or missing (n = 3), although a criterion of inclusion of DTC was a size above 10 mm.

Our study confirmed the value of ultrasonographic criteria to detect malignant lesions preoperatively. A previous ultrasound analysis of 550 consecutive patients, who underwent a total thyroidectomy for one or more nodules, found a positive correlation between hypoechoic features and malignant lesions, but the presence of microcalcifications was still more useful, with a positive predictive value of 94% vs 57.8% for the former characteristic [40]. Many benign lesions are hypoechoic [42]. The presence of irregular margins and central microcalcifications are independent risk factors of malignancy. Papini tried to correlate ultrasound data to cytology in 402 subjects with a nodule measuring 8 to 15 mm. Irregular margins (RR = 16.83, P = 0.001), central blood flow (RR = 14.3, P = 0.0011) and presence of microcalcifications (RR = 4.97, P = 0.05) were independent predictive factors of malignancy [43]. A prospective study in 188 patients with a thyroid disease including 37 DTC, found a higher risk of cancer in the group of solitary nodules when central calcifications were present (55% vs 23%, P = 0.016). Compared to the non-calcified MNG, the calcified solitary nodule was associated with a relative risk of cancer of 22.8 (95% CI, 5.4–95.5). [44]. Another study found among patients with solitary nodules, a frequency of 75.7% of DTC, when ultrasonography had shown microcalcifications [45]. We could not analyse the blood flow type of the lesions, as the sample of patients who had a Doppler in addition to the ultrasonographic analysis, was too small. The main problem was the difficulty to model the risk by a mathematical formula, due to the small size of our sample and the presence of missing data.

Preoperative TSH level alone is certainly not sufficient to know if the nodule is malignant or benign, as the threshold of significance has a specificity and sensitivity of only 34% and 66% respectively. But, it might be of interest to create a prediction model including the level of TSH, the presence of irregular margins, central microcalcifications, central blood flow and results of elastography to enhance the specificity and sensitivity.

5. Conclusion

Our study is retrospective and the sample of patients is probably too small to conclude, but it confirmed that an elevated level of TSH is an independent risk factor of DTC, even if it remains in the normal range. Hypothyroidism could be responsible for the development of DTC, and it might be necessary to adapt the treatment of autoimmune thyroiditis, to obtain TSH values within the lower end of the normal range, to avoid or to reduce the development of DTC.
Disclosure of interest

The authors declare that they have no conflicts of interest concerning this article.

References


