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Calcitonin Levels in Thyroid Disease Are Not Affected by Autoimmune Thyroiditis or Differentiated Thyroid Carcinoma

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Keywords
Autoimmune thyroiditis · Calcitonin · Medullary thyroid cancer · Differentiated thyroid cancer

Abstract
Introduction: Association between hypercalcitoninemia and pathological conditions such as autoimmune thyroiditis (AIT) or differentiated thyroid carcinoma (DTC) has been addressed, with conflicting results. We evaluated the prevalence and the clinical relevance of elevated basal serum calcitonin (CT) levels in non-neoplastic (nodular goiter [NG] and AIT) and neoplastic thyroid diseases (DTC). ***Methods:*** We retrospectively evaluated 3,250 consecutive patients with thyroid nodular disease who underwent fine-needle aspiration cytology with adequate sample. After exclusion of medullary thyroid cancer (MTC) patients were divided according to the presence/absence of thyroid autoimmunity into NG or nodular autoimmune thyroiditis (N-AIT) and, according to cytological results, in benign or suspicious/malignant nodules. ***Results:*** One hundred ninety-seven/3,250 patients (6.0%) showed CT level >10 pg/mL. In 11/3,250 (0.3%) cases, a final histological diagnosis of MTC was made, while the remaining 186/3,250 patients (5.7%) had non-MTC-related hypercalcitoninemia (CT > 10 pg/mL). According to cytological diagnosis, the rate of hypercalcitoninemia was similar in class II and class V–VI groups (5.4 vs. 6.9%, $p = 0.4$). The occurrence of hypercalcitoninemia was significantly higher in patients with NG (166/2,634 [6.3%]) than in patients with N-AIT (20/605 [3.3%]) ($p = 0.004$). However, after matching by sex, no difference was found between the 2 groups (NG and N-AIT). These results were confirmed in 598 patients submitted to surgery. ***Conclusions:*** AIT and DTC seem not to affect serum CT levels in patients with thyroid nodules. Therefore, hypercalcitoninemia, in these patients, should be submitted to the same diagnostic workup than patients without AIT or DTC.

Introduction

Calcitonin (CT), a 32-amino acid calcium-lowering peptide secreted by the C-cells (parafollicular cells) of the thyroid, is used as a marker for the diagnosis and the follow-up of medullary thyroid cancer (MTC) [1]. In pa-

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tients with a nodular thyroid, the measurement of basal serum CT levels has been proposed as a systematic screening method for MTC [2, 3]. The routine measurement of CT in patients with thyroid nodules can be useful in the early diagnosis of MTC and C-cell hyperplasia (CCH) and several studies suggested that a cutoff of 10 pg/mL had a very high negative predictive value in spite of a low positive predictive value, with a high rate of false-positive results [4–10]. Basal CT levels >100 pg/mL are widely considered an indication for surgery [1], while the management of patients showing a mild increase in basal serum CT levels appears to be more controversial [10]. Indeed, the elevation of basal serum CT levels has also been associated with clinical factors (i.e., sex, age, and cigarette smoking) [11–14], pathological conditions (i.e., small-cell lung carcinoma, chronic renal failure, hypergastrinemia, neuroendocrine tumors, autoimmune thyroiditis (AIT), and micropapillary thyroid carcinoma) [15, 16] and pharmacological agents (i.e., proton-pump inhibitor, glucocorticoids, and β-blockers) [17, 18]. Consequently, while the reported prevalence of MTC among patients with thyroid nodules is low (0.26–1.30%), the frequency of elevated basal CT levels reported in these patients is much higher (0.6–6.8%) [4–10].

The association between AIT and hypercalcitoninemia is still controversial. Some authors have reported CCH in some thyroid specimens affected by Hashimoto's thyroiditis [19], while others have reported decreased basal serum CT levels that are probably caused by atrophy, fibrosis, and destruction of both follicular and C-cells [15]. Grani et al. [15] retrospectively evaluated the association between hypercalcitoninemia and the presence of anti-TPO antibodies in 1,073 patients with thyroid nodules. The prevalence of patients with a basal serum CT value >10 pg/mL, considered as "suspicious", was not significantly different between patients with thyroid autoimmunity and those without (3.9 vs. 3.0%).

Differentiated thyroid carcinoma (DTC) has been reported to be associated with CCH and high levels of basal CT [18]. It has been proposed that these tumors may release substances that have a paracrine stimulatory action on the C-cells and eventually lead to an increase of serum CT levels. In this regard, Verga et al. [20] analyzed the prevalence and the histological patterns of CCH in 15 patients with multinodular goiter and hypercalcitoninemia. They found that in 20% of them, the histological diagnosis of DTC was associated with CCH. Therefore, they speculated the possible over expression in thyroid cancer of paracrine growth factors influencing the surrounding C-Cell. Nevertheless, currently the possible association between hypercalcitoninemia and DTC is still debated [16]. Therefore, the aim of our study was to evaluate the prevalence and the clinical relevance, especially in terms of utility in MTC detection, of elevated basal serum CT levels in non-neoplastic (nodular goiter (NG) and AIT) and neoplastic thyroid diseases (DTC).

Materials and Methods

Study Population

We retrospectively evaluated 3,250 (2,546 females and 704 males, mean age 57 ± 14 years, range 8–94 years) consecutive patients with thyroid nodular disease who underwent fine-needle aspiration cytology (FNAC) with adequate cytology from 2003 to 2019. Exclusion criteria were (1) patients with a family history of MTC or MEN syndrome; (2) patients with known presence of kidney failure, hyperparathyroidism, chronic atrophic gastritis, neuroendocrine tumor or lung cancer; (3) patients without serum CT measurement at the time of FNAC.

All patients were submitted to biochemical evaluation (TPOAb, TgAb, FT4, FT3, TSH, and CT) and neck ultrasonography (US). At the neck ultrasound, 2,580/3,250 (79.4%) patients had multiple NG while 670/3,250 (20.6%) had a solitary nodule. The mean diameter of nodules was 25.3 ± 13.4 mm. According to Bethesda classification [21], FNAC was benign (class II) in 2,788/3,250 (85.8%) patients, atypia of undetermined significance/follicular lesion of undetermined significance (AUS/FLUS)-follicular neoplasm/suspicious for follicular neoplasm (FN/SFN) (class III–IV) was found in 264/3,250 (8.2%) patients, while suspicious for malignancy (class V) and malignant (class VI) cytologies were observed in 48/3,250 (1.5%) patients and in 145/3,250 (4.5%) patients, respectively. Cytological diagnosis of MTC was reported in 5/3,250 (0.1%) patients.

Based on clinical, biochemical, and ultrasound data available at the time of FNAC, patients with thyroid nodules were classified into 2 groups: (1) nodular autoimmune thyroiditis (N-AIT) in the presence of positivity of anti-thyroid antibodies, defined by TgAb and/or TPOAb ≥100 UI/L, with or without a "thyroiditis" pattern on neck US (diffusely hypoechoic echogenicity, parenchymal heterogeneity, and echogenic septation) and with or without hypothyroidism. Patients with a "thyroiditis" pattern on neck US but with a mild increase of TgAb and/or TPOAb (<100 UI/L) with or without hypothyroidism were also considered as affected by N-AIT; (2) the diagnosis of NG was made in the presence of negative anti-thyroid antibodies, no "thyroiditis" pattern on US or in the presence of mild positive anti-thyroid antibodies (TgAb and/or TPOAb <100 UI/L) without "thyroiditis" pattern on US. Six hundred seven patients/3,250 (18.7%) were classified in the N-AIT group and 2,643/3,250 (81.3%) in the NG group.

Methods

Serum TSH was measured by chemiluminescent immunometric assay (Immulite 2000; DPC Diagnostic Products Corporation, Los Angeles, CA, USA). Measurement of TgAb and TPOAb was

performed with a solid-phase, enzyme-labeled, chemiluminiscent sequential immunometric assay (Immulite 2000; Siemens Medical Solutions Diagnostics, Los Angeles, CA, USA). TgAb values ≤45 IU/mL and TPOAb ≤35 IU/mL were considered to be negative, while values ≥100 for both antibodies were considered to be clearly positive ([2-fold the upper normal level, with a higher sensitivity for Hashimoto thyroiditis diagnosis] [22]). Histological thyroiditis was defined on the basis of the histological report, irrespective of diffuse or focal lymphocitic thyroiditis.

From 2003 to 2019, serum CT was measured in the same laboratory, by 2-site immunoradiometric assay with analytic sensitivity of 1.5 pg/mL and functional sensitivity of 4 pg/mL, without differences in CT range regarding sex (IRMA-hCT Cisbio Bioassays – Parc Marcel Boiteux, Codolet France). Patients with basal CT levels >5 and >10 pg/mL were considered to have hypercalcitoninemia.

Neck US was performed by experienced endocrinologists (members of our staff) using a high resolution ultrasound color Doppler apparatus (AU 590 Asynchronous; Esaote Biomedica, Firenze, Italy) with a 7.5-MHz linear transducer. The anteroposterior (AP), transverse (T), and longitudinal (L) diameters of each nodule were measured by US. The volume of nodules was calculated using the ellipsoid formula ($AP \times T \times L \times \pi/6$) ($cm^3$). FNAC was performed under ultrasound guidance using a 23/25-gauge needle and only nodules clearly distinct from the surrounding parenchyma were submitted to FNAC. The material was air dried, stained with May-Grunwald Giemsa and interpreted by an experienced cytologist. Cytological results were reported according to the criteria of the 2017 Bethesda classification for reporting thyroid cytopathology [21]: benign (class II), AUS/FLUS-FN/SFN (class III–IV), suspicious for malignancy (class V), and malignant (class VI) nodules. Patients with multinodular goiter were given the cytological category with the highest risk (class II < class III–IV < class V–VI). A written informed consent was obtained from all study patients and the study was conducted in accordance with the principles set out in the Declaration of Helsinki.

Statistical Analysis

Epidemiological data are presented as the mean ± SD and median when needed. The *t* test for independent data or the Mann-Whitney test were performed for normally or non-normally distributed variables, respectively. To evaluate significant differences in data frequency we analyzed contingency tables. Tables with size larger than 2 × 2 were examined by the χ^2 test or a numerical approximation of the Fisher's exact test, when all cell frequencies were >4 or not, respectively. To evaluate the impact of thyroid cancer on serum CT levels in the cytological series, patients with indeterminate cytology (class III–IV) were excluded from the analysis and patients with suspicious or malignant cytology (class V–VI) were grouped. Linear regression models were built to examine relationship between CT values and thyroid volume. We performed a multivariate analysis using a stepwise procedure based on the Akaike criterion to identify variables with independent prognostic significance for serum CT levels and to calculate the odds ratio. Statistical analysis was performed using the software StatView for Windows version 5.0.1 (SAS Institute, Cary, NC, USA) and the SPSS Statistics version 22.0. A p value <0.05 was considered statistically significant.

Results

Hypercalcitoninemia and Medullary Thyroid Carcinoma

One hundred ninety-seven/3,250 patients (6.0%) showed a basal CT level >10 pg/mL. Among patients with hypercalcitoninemia, 54/197 (27.4%) were submitted to surgery. In 11/54 (20.3%), a histological diagnosis of MTC was made. In the remaining 186 patients, the diagnosis of MTC was excluded on the basis of histological results (n = 43/186, 23.1%) or additional test (n = 143/186, 76.9%) such as stimulating test (calcium gluconate administration or pentagastrin test) and/or CT measurement in fine-needle aspirate washout fluid of the suspicious nodules. In the subgroup of patients with hypercalcitoninemia without MTC (n = 43), the main indication for surgery was a large goiter in 19/43 (44.2%), suspicious/malignant cytology in 12/43 (27.9%), class III–IV cytology in 8/43 (18.6%), and CT levels suspicious for MTC in 4/43 (9.3%). Basal serum CT levels in MTC patients were 915.3 ± 701.0 pg/mL (range 12–1,992 pg/mL, median 923 pg/mL) significantly higher than that observed in patients without MTC (mean 14.6 ± 7.3 pg/mL [range 10.1–57.9 pg/mL, median 11.8 pg/mL]) (p < 0.0001).

Hypercalcitoninemia without Medullary Thyroid Carcinoma

Patients with MTC were excluded from the statistical analysis and the final study group included 3,239 patients (Table 1). The mean value of serum CT and the rate of patients with hypercalcitoninemia (defined as serum CT levels >5 or >10 pg/mL) were significantly higher in males than females (p < 0.0001). In 186/3,239 (5.7%) patients with basal serum CT levels >10 pg/mL, the mean CT value was 14.6 ± 7.3 pg/mL (range 10.1–57.9 pg/mL, median 11.8 pg/mL) and CT levels were significantly higher in men (mean 16.1 ± 9.3, range 10.1–57.9 pg/mL, median value 12.2 pg/mL) than in females (mean 13.1 ± 4.0, range 10.1–31.6 pg/mL, median value 11.7 pg/mL) (p < 0.0001). The thyroid volume was significantly larger in males than females (median volume 31 mL in men vs. 20 mL in females, p < 0.0001). A significant correlation between basal CT value and thyroid volume was observed (p < 0.0001).

Hypercalcitoninemia and Differentiated Thyroid Cancer (DTC)

Cytological Results

To evaluate the association between serum CT values and thyroid nodule cytology, we classified our patients ac-

Table 1. Epidemiological, clinical, and biochemical features of the cohorts (MTC excluded)

	Total group	Male	Female	*p* value (M vs. F)
N (%)	3,239	700 (21.6%)	2,539 (78.4%)	
Age (yr)				$p < 0.0001$[a]
Median	59	61	58	
Mean ± SD	57.1±14	59.5±13.3	56.5±14	
Range	8–94	16–89	8–94	
Hyper-CT (cutoff 5 pg/mL),[b] *n* (%)	769 (23.7)	300 (42.8)	469 (18.4)	$p < 0.0001$[c]
Hyper-CT value (>5 pg/mL)				$p < 0.0001$[d]
Median	7.9	8.4	7.6	
Mean ± SD	8.9±5.0	10.0±6.7	8.2±3.2	
Range	5.0–57.9	5.0–57.9	5.0–31.6	
Hyper-CT (cutoff 10 pg/mL), *n* (%)	186 (5.7)	95 (13.5)	91 (3.6)	$p < 0.0001$[c]
Hyper-CT value (>10 pg/mL)				$p < 0.0001$[d]
Median	11.8	12.2	11.7	
Mean ± SD	14.6±7.3	16.1±9.3	13.1±4	
Range	10.1–57.9	10.1–57.9	10.1–31.6	
Thyroid gland volume, mL				$p < 0.0001$[d]
Median	25	31	20	
Mean ± SD	35.1±30.4	42.6±34.9	27.1±22.2	
Range	6–240	9–240	6–127	

MTC, medullary thyroid cancer; N-AIT, nodular autoimmune thyroiditis; Hyper-CT, hypercalcitoninemia (>10 pg/mL); CT, calcitonin (in patients with serum CT, levels ≥10 pg/mL). [a] ANOVA test. [b] According to functional sensitivity of our assay. [c] χ^2 test. [d] Mann-Whitney test.

cording to the 2017 Bethesda classification for reporting thyroid cytopathology [21]. To investigate the correlation between serum CT levels and the cytological diagnosis of thyroid cancer, patients were classified into 2 groups: benign (Class II: $n = 2,786$) and suspicious/malignant cytology (Class V–VI, $n = 189$). Patients with Class III–IV cytology ($n = 264$) were excluded from the analysis.

The prevalence of hypercalcitoninemia defined according to the cutoff of 10 pg/mL, was similar in class II and class V–VI groups (150/2,786, 5.4% vs. 13/189, 6.9%, $p = 0.4$), even when males (76/578, 13.1% vs. 8/53, 15%, $p = 0.6$) and female (74/2,208, 3.3% vs. 5/136, 3.6%, $p = 0.8$) were analyzed separately (Table 2). Similarly, no differences in the rate of hypercalcitoninemia were also observed when a lower cutoff (5 pg/mL) of calcitonin was used (Table 2). Moreover, serum CT levels were not different between patients with class II and class III–IV cytology, both in the total group than in males and females (Table 2).

Histological Results

The potential correlation between serum CT values and DTC was also evaluated in a subgroup of patients submitted to thyroidectomy ($n = 598/3,239$, 18.5%) (Table 3). Benign histology was reported in 353/598 patients (59.0%). In 240/598 patients (40.3%), a histological diagnosis of DTC was made. Papillary thyroid cancer was diagnosed in 228/241 (94.6%) and follicular thyroid cancer in 13/241 (5.4%) patients. At last, in 4/598 (0.7%) patients, a diagnosis of CCH was made. All patients with a histological diagnosis of CCH had hypercalcitoninemia, while no differences in the rate of hypercalcitoninemia, defined according to the cutoff of 10 pg/mL, was observed between benign histology (19/353, 5.4%) and DTC (21/241, 8.7%, $p = 0.13$). Similarly, no differences in the prevalence of hypercalcitoninemia and in the serum CT levels were found when the same analysis was performed according to sex (Table 3). Using a lower serum CT cutoff (5 pg/mL), no differences in the rate of hypercalcitoninemia was observed between benign and malignant nodules both in the total group ($p = 0.55$), in males (0.86) and females ($p = 0.31$) subgroups (Table 3). Moreover, serum CT levels were not different between patients with benign nodules and DTC, in the total group, in males and females (Table 3).

Hypercalcitoninemia and AIT

Clinical and Biochemical Diagnosis

As reported in Table 4, 2,634/3,239 (81.3%) patients were classified as having NG and 605/3,239 (18.7%) pa-

Table 2. Hypercalcitoninemia prevalence and basal serum CT levels according to cytological diagnosis (class III–IV excluded, *n* = 264)

	Bethesda classification		*p* value
	class II	class V–VI	
Total group, *n* (%), *n* = 2,985	2,786 (93.6)	189 (6.4)	
Hyper-CT (cutoff 5 pg/mL),[a] *n* (%)	641 (22.9)	46 (24.3)	0.79[b]
Hyper-CT value (>5 pg/mL)			0.63[c]
Median	7.9	7.8	
Mean ± SD	8.9±4.9	9.5±7.3	
Range	5.0–57.9	5.1–53.4	
HyperCT (cutoff 10 pg/mL), *n* (%)	150 (5.4)	13 (6.9)	0.4[b]
Hyper-CT value (>10 pg/mL)			0.6[c]
Median	11.9	11.8	
Mean ± SD	14.8±7.3	15.8±11.7	
Range	10.1–57.9	10.2–53.4	
Female, *n* (%)	2,208 (94.2)	136 (5.8)	
Hyper-CT (cutoff 5 pg/mL),[a] *n* (%)	641 (18.3)	25 (18.3	1.0[b]
CT value, pg/mL			0.47[c]
Median	7.6	7.7	
Mean ± SD	8.1±3.2	8.4±3.2	
Range	5.0–31.6	5.1–21	
Hyper-CT (cutoff 10 pg/mL), *n* (%)	74 (3.3)	5 (3.6)	0.8[b]
Hyper-CT value (>10 pg/mL)			0.9[c]
Median	11.8	11.6	
Mean ± SD	13.1±4.0	13.1±4.4	
Range	10.1–31.6	10.3–21.0	
Male, *n* (%)	578 (91.6)	53 (8.4)	
Hyper-CT (cutoff 5 pg/mL),[a] *n* (%)	247 (42.7)	21 (39.6)	0.77[b]
CT value, pg/mL			0.83[c]
Median	8.5	7.9	
Mean ± SD	10.1±6.7	10.8±10.2	
Range	5.0–57.9	5.2–53.4	
Hyper-CT (cutoff 10 pg/mL), *n* (%)	76 (13.1)	8 (15)	0.6[b]
Hyper-CT value (>10 pg/mL)			0.7[c]
Median	12.2	12.0	
Mean ± SD	16.5±9.1	17.5±14.7	
Range	10.1–57.0	10.2–53.0	

Hyper-CT, hypercalcitoninemia; CT, calcitonin. [a] According to functional sensitivity of our assay. [b] χ^2 test. [c] Mann-Whitney test.

tients as having N-AIT. The prevalence of hypercalcitoninemia defined according to the cutoff of 10 pg/mL was significantly higher in patients with NG (166/2,634 [6.3%]) than patients with N-AIT (20/605 [3.3%]) (p = 0.004). When the analysis was performed according to gender, no difference between NG and N-AIT was found both in males (p = 0.76) and females (p = 0.08). Similarly, no differences in the rate of hypercalcitoninemia were also observed when a lower cutoff (5 pg/mL) of calcitonin was used (Table 4). Moreover, the serum CT levels were not different between patients with NG and N-AIT both in the total group than in males and females (Table 4).

The same analysis was performed according to the presence or absence of TPOAb using a cutoff of >100 IU/mL. TPOAb were clearly positive (≥100 IU/mL, TPOAb+ group) in 440/3,239 (13.6%) patients and mild positive (<100 IU/mL) or negative (<45 IU/mL) in 2,799/3,239 (86.4%) patients (TPOAb− group). The prevalence of hypercalcitoninemia defined according to the cutoff of 10 pg/mL did not differ between the 2 groups of patients (18/440 [4.1%] in TPOAb+ group vs. 168/2,799 [6.0%] in TPOAb− group, p = 0.1). Similar results were observed also when considering only males (7/44 [15.9%] in TPOAb+ group vs. 88/656 [13.4%] in TPOAb− group,

Table 3. Hypercalcitoninemia prevalence and basal serum CT levels according to histological diagnosis in 598/3,239 patients submitted to thyroidectomy (CCH patients were excluded, $n = 4$)

	Histological results		*p* value
	benign	DTC	
Total group, *n* (%), *n* = 594	353 (59.4)	241 (40.5)	
Hyper-CT (cutoff 5 pg/mL),[a] *n* (%)	81 (22.9)	61 (25.3)	0.55[b]
Hyper-CT value (>5 pg/mL)			0.18[c]
Median	8.0	8.3	
Mean ± SD	9.1±6.3	10.9±9.7	
Range	5.1–57.9	5.1–64.5	
Hyper-CT (cutoff 10 pg/mL), *n* (%)	19 (5.4)	21 (8.7)	0.13[b]
Hyper-CT value (>10 pg/mL)			0.86[c]
Median	12.4	12.2	
Mean ± SD	15.6±10.6	18.0±14.2	
Range	10.2–57.9	10.1–64.5	
Female, *n* (%)	260 (59.6)	176 (40.4)	
Hyper-CT (cutoff 5 pg/mL),[a] *n* (%)	43 (16.5)	36 (20.4)	0.31[b]
Hyper-CT value (>5 pg/mL)			0.18[c]
Median	7.6	8.3	
Mean ± SD	8.4±3.4	10.6±9.9	
Range	5.1–19.7	5.1–64.5	
Hyper-CT (cutoff 10 pg/mL), *n* (%)	10 (3.8)	10 (5.6)	0.48[b]
Hyper-CT value (>10 pg/mL)			0.93[c]
Median	12.3	11.8	
Mean ± SD	13.7±3.0	19.0±16.5	
Range	11–19.7	10.1–64.	
Male, *n* (%)	93 (58.8)	65 (41.1)	
Hyper-CT (cutoff 5 pg/mL),[a] *n* (%)	38 (40.9)	25 (38.5)	0.86[b]
Hyper-CT value (>5 pg/mL)			0.51[c]
Median	8.2	7.9	
Mean ± SD	9.8±8.5	11.2±9.7	
Range	5.1–57.9	5.2–53.4	
Hyper-CT (cutoff 10 pg/mL), *n* (%)	9 (9.6)	11 (16.9)	0.22[b]
Hyper-CT value (>10 pg/mL)			0.79[c]
Median	12.4	12.4	
Mean ± SD	17.8±15.2	17.1±12.4	
Range	10.2–57.9	10.2–53.4	

CCH, C-cell hyperplasia; Hyper-CT, hypercalcitoninemia; DTC, differentiated thyroid carcinoma; CT, calcitonin. [a] According to functional sensitivity of our assay. [b] χ^2 test. [c] Mann-Whitney test.

$p = 0.6$) and in females (11/396 [2.8%] in TPOAb+ group vs. 80/2,143 [3.7%] in TPOAb− group, $p = 0.3$). No differences in the rate of hypercalcitoninemia were also observed when a lower cutoff (5 pg/mL) was used. Moreover, serum CT levels were not different between TPOAb+ and TPOAb− group, both in the total group than in males and females (Table 4).

Histological Results

The potential correlation between CT and AIT was also evaluated in a subgroup of patients with non-MTC hypercalcitoninemia submitted to thyroidectomy ($n = 598/3,239$, 18.5%). Thyroiditis was found at histology in 127/598 (21.2%) patients. The prevalence of hypercalcitoninemia, defined according to the cutoff of 10 pg/mL, did not differ between patients with (7/127 [5.5%]) and without histological thyroiditis (37/471 [7.8%]) ($p = 0.44$). As well, hypercalcitoninemia was not related to the presence of histological thyroiditis also when the analysis was performed according to sex ($p = 0.07$ and $p = 0.4$ in males and females, respectively). No differences in the rate of hypercalcitoninemia were also observed when a lower

Table 4. Hypercalcitoninemia prevalence and basal serum CT levels according to clinical diagnosis

	Clinical diagnosis		*p* value
	NG	N-AIT	
Total group, *n* (%), *n* = 3,239	2,634 (81.3)	605 (18.7)	
Hyper-CT (cutoff 5 pg/mL),[a] *n* (%)	646 (24.5)	123 (20.3)	0.02[b]
Hyper-CT value (>5 pg/mL)			0.06[c]
Median	8.1	7.6	
Mean ± SD	9.0±5.2	8.2±3.5	
Range	5.0–57.9	5.0–24.3	
Hyper-CT (cutoff 10 pg/mL), *n* (%)	166 (6.3)	20 (3.3)	0.004[b]
Hyper-CT value (>10 pg/mL)			0.66[c]
Median	11.8	11.6	
Mean ± SD	14.7±7.5	14.0±5.0	
Range	10.1–57.9	10.2–24.3	
Female, *n* (%)	1,991 (78.4)	548 (21.6)	
Hyper-CT (cutoff 5 pg/mL),[a] *n* (%)	368 (18.4)	101 (18.4)	1.0[b]
Hyper-CT value (>5 pg/mL)			0.2[c]
Median	7.7	7.3	
Mean ± SD	8.3±3.3	7.9±3.1	
Range	5.0–31.6	5.1–23.5	
Hyper-CT (cutoff 10 pg/mL), *n* (%)	78 (3.9)	13 (2.4)	0.08[b]
Hyper-CT value (>10 pg/mL)			0.45[c]
Median	11.6	12.6	
Mean ± SD	13.0±3.9	13.9±4.5	
Range	10.1–31.9	10.3–23.5	
Male, *n* (%)	643 (91.9)	57 (8.1)	
Hyper-CT (cutoff 5 pg/mL),[a] *n* (%)	278 (43.2)	22 (38.6)	0.57[b]
Hyper-CT value (>5 pg/mL)			0.89[c]
Median	8.4	8.2	
Mean ± SD	10.1±6.9	9.5±4.8	
Range	5.0–57.9	5.0–24.3	
Hyper-CT (cutoff 10 pg/mL), *n* (%)	88 (13.7)	7 (12.3)	0.76[b]
Hyper-CT value (>10 pg/mL)			0.55[c]
Median	12.2	10.8	
Mean ± SD	16.3±9.4	14.1±6.2	
Range	10.1–57.9	10.2–24.3	

N-AIT, nodular autoimmune thyroiditis; NG, nodular goiter; Hyper-CT, hypercalcitoninemia, CT, calcitonin.
[a] According to functional sensitivity of our assay. [b] χ^2 test. [c] Mann-Whitney test.

cutoff (5 pg/mL) was used (Table 5). Moreover, serum CT levels were not different between patients with (7/127 [5.5%]) and without histological thyroiditis, both in the total group than in males and females groups (Table 5).

Follow-Up Data in Patients with Hypercalcitoninemia not Submitted to Surgery at the Time of the First Evaluation

Among 143 patients with non-MTC-related hypercalcitoninemia and not submitted to surgery, long-term follow-up data (median follow-up of 9.9 years) were available only in 59/143 (41.0%) patients. Four out of 59 (6.7%) patients were submitted to surgery during follow-up: in 1 patient submitted to surgery because of a positive pentagastrin test, a CCH was found at histology, while in another patient, MTC <1 cm (micro-MTC) was found. In this patient, surgery was performed for the presence of large cytologically benign nodule and previous external beam radiotherapy for laryngeal cancer. Therefore, we were not able to establish the real prevalence of MTC in this group because surgery was not performed in all patients.

Repeated measurement of basal CT during follow-up was available in 27/59 (45.7%) patients. Serum CT be-

Table 5. Hypercalcitoninemia prevalence and basal serum CT levels, according to histological presence/absence of AIT in 598/3,239 patients submitted to total thyroidectomy

	Histological diagnosis		*p* value
	AIT	No-AIT	
Total group, *n* (%), *n* = 598	127 (21.2)	471 (78.8)	
Hyper-CT (cutoff 5 pg/mL),[a] *n* (%)	26 (20.4)	120 (25.4)	0.29[b]
Hyper-CT value (>5 pg/mL)			0.65[c]
Median	7.9	8.3	
Mean ± SD	9.1±4.2	10.5±9.0	
Range	5.1–23.5	5.1–64.5	
Hyper-CT (cutoff 10 pg/mL), *n* (%)	7 (5.5)	37 (7.8)	0.44[b]
Hyper-CT value (>10 pg/mL)			0.90[c]
Median	12.6	12.4	
Mean ± SD	14.7±4.7	18.3±13.2	
Range	10.3–23.5	10.4–64.5	
Female, *n* (%)	108 (24.7)	329 (75.3)	
Hyper-CT (cutoff 5 pg/mL),[a] *n* (%)	20 (18.5)	60 (18.2)	1.0[b]
Hyper-CT value (>5 pg/mL)			0.73[c]
Median	7.8	8.1	
Mean ± SD	9.5±4.7	9.6±8.0	
Range	5.1–23.5	5.1–64.5	
Hyper-CT (cutoff 10 pg/mL), *n* (%)	7 (6.4)	14 (4.2)	0.4[b]
Hyper-CT value (>10 pg/mL)			0.94[c]
Median	12.6	11.9	
Mean ± SD	14.7±4.7	17.8±14.0	
Range	10.3–23.5	10.1–64.5	
Male, *n* (%)	19 (11.8)	142 (88.2)	
Hyper-CT (cutoff 5 pg/mL),[a] *n* (%)	6 (31.6)	60 (42.2)	0.46[b]
Hyper-CT value (>5 pg/mL)			0.32[c]
Median	7.9	8.8	
Mean ± SD	7.6±1.4	11.1±9.8	
Range	5.2–9.3	5.1–57.9	
Hyper-CT (cutoff 10 pg/mL), *n* (%)	0 (0)	23 (16.2)	0.07[b]
Hyper-CT value (>10 pg/mL)			NA
Median	NA	12.7	
Mean ± SD	NA	18.6±13.0	
Range	NA	10.2–57.9	

AIT, autoimmune thyroiditis; Hyper-CT, hypercalcitoninemia; CT, calcitonin; NA, not applicable. [a] According to functional sensitivity of our assay. [b] χ^2 test. [c] Mann-Whitney test.

came undetectable (<10 pg/mL) in 17/27 (63%) patients and remained stable in the remaining 10 cases. We observed significant lower serum CT values in the subgroup of patients in whom serum CT became undetectable (median 11 pg/mL, range 10.4–41 pg/mL) rather than those in which serum CT values remained stable or increased during the follow-up (median 24.7 pg/mL, range 10.9–42). In 41/57 (72%) patients, routine neck ultrasound examination was performed and only in 6/41 (14.6%) patients, a small increase of thyroid nodule size was observed.

Risk Factors Associated with Hypercalcitoninemia

We investigated, by multivariate analysis, several clinical parameters (sex, age, presence/absence of AIT, cytological and histological results, and thyroid volume) as possible risk factors for hypercalcitoninemia. To perform this analysis, we used serum CT as a continuous variable and also as a categorical variable (detectable or undetectable according to the 2 cutoffs used in the study). The only variable associated with serum CT levels was male sex. In particular, CT values in male patients were 2.824 pg/mL greater than females (p =

0.000), and male sex was associated with an odds ratio of 4.595 for hypercalcitoninemia (95% CI 3.313–6.383, $p = 0.000$).

Discussion

The routinely measurement of CT in patients with thyroid nodules can be useful in the early diagnosis of MTC and CCH [2–9] and CT levels >100 pg/mL are widely considered an indication for surgery [10]. On the contrary, the clinical significance of a mild increase of serum CT levels in patients with thyroid nodules appear to be more controversial. In the literature, the reported prevalence of hypercalcitoninemia not associated with MTC, range from 0.6 to 6.8% [10]. In our series, the overall prevalence of MTC was 0.3%, while the occurrence of non-MTC-related hypercalcitoninemia was 5.7%.

Several clinical and pathological factors may induce an increase of serum CT levels in patients with nodular disease without MTC. Among thyroid diseases, both DTC and AIT have been reported to be associated with hypercalcitoninemia but without conclusive results [15, 16, 18, 19]. To date, the potential correlation between the presence of hypercalcitoninemia and DTC has been poorly explored. To our knowledge, this association has been investigated in only 1 study including 494 patients with thyroid nodules, submitted to surgery: the prevalence of hypercalcitoninemia was 1.6% in patients with PTC and 1.4% in patients without PTC at histology, with no significant difference between the 2 groups [16]. Our results, obtained from a large cohort of consecutive patients, are in agreement with the results reported by Rosario and Calsolari [16] and do not support any association between hypercalcitoninemia and cytological results. To confirm our findings, we repeated the analysis in a subgroup of patients submitted to surgery and, similarly, we did not find any difference in the prevalence of hypercalcitoninemia between DTC and benign goiter (8.7 vs. 5.4%, $p = 0.13$).

On the contrary, several studies evaluated the relationship between hypercalcitoninemia and AIT with conflicting results [15–17, 19]. Some authors reported the presence of CCH in some thyroid specimens with Hashimoto's thyroiditis [19], while others reported decreased serum CT values probably caused by atrophy, fibrosis, and destruction of both follicular and C-cells [15]. Recently, Ito et al. [23] found a significant association between ECLIA-CT and TgAb titer: ECLIA-CTs were statistically lower in the TgAb positive group than in the TgAb negative group suggesting that CT level might be affected by thyroiditis.

We found a significant increase of hypercalcitoninemia prevalence in patients with NG with respect to N-AIT patients. However, when we analyzed our data according to gender, no difference was found between NG and N-AIT patients. We believe that the difference observed in the whole group is probably due to the higher number of females in the group of N-AIT. As a matter of fact, we found significantly lower serum CT levels in females compared to males and consequently lower prevalence of hypercalcitoninemia in females than man. Since the thyroid volume was significantly larger in males than females, we hypothesized a possible association between thyroid volume and CT levels. We found a correlation in patients with thyroid nodular disease between thyroid volume and basal serum CT levels too, in agreement with previous studies [11, 13, 24]. Also in a subgroup of patients submitted to thyroidectomy, the presence of AIT at histology was not associated with a higher prevalence of hypercalcitoninemia. No association between N-AIT and hypercalcitoninemia was also observed when the diagnosis of the autoimmune disease was made based on TPOAb levels. Our results are similar to those reported by Rosario and Calsolari [16] and Grani et al. [15]; however, differently to our study, these authors defined the presence of AIT only based on the positivity of anti-thyroid antibodies without histological or clinical data. Based on follow-up data, surgery was indicated in 4/59 (6.8%) of cases. We found an incidental micro-MTC in only 1 patient and, in another one, a CCH. Our results are similar to those reported by Costante et al. [9] in a subgroup of 171 patients with a slight increase of basal CT values (>10 and <20 pg/mL) in which only 1 patient underwent surgery because of a positive pentagastrin test with histological diagnosis of CCH. In our series of patients with hypercalcitoninemia, we observed a high rate of patients in whom serum CT became undetectable during follow-up. These results were probably due to the inclusion in the study group of patients with a slight increase of serum CT levels. Indeed, we found a significant correlation between basal serum CT level and its trend during the follow-up. Significantly lower levels of basal serum CT were observed in the subgroup of patients in whom serum CT became undetectable (median 11 vs. 24.7 pg/mL). In addition, since not all patients with hypercalcitoninemia were submitted to additional serum CT measurement during the follow-up, a possible selection bias might have occurred in our study.

Some limitations of this study are intrinsic to its retrospective design, in particular histological results to rule

out MTC were not available in all patients. However, the findings from a subgroup of patients submitted to surgery confirm the lack of association between hypercalcitoninemia and both AIT and DTC, as already observed in the cytological series.

On the other hand, the study has several strengths, including a similar diagnostic approach and follow-up strategies in the same institute. In addition, our cohort of patients is the largest, to our knowledge, in which the clinical impact of AIT and DTC on basal serum CT levels has been evaluated in both cytological and histological series.

Conclusion

Thyroid autoimmune disease and DTC seem not to affect basal serum CT levels in patients with thyroid nodules. Therefore, in the presence of hypercalcitoninemia, MTC should be routinely excluded also in the presence of AIT or a cytological diagnosis of DTC. In addition, our data suggest that an aggressive approach may not be necessary in patients with only a slight increase of basal CT. We believe that an adequate follow-up including serum CT measurement and thyroid ultrasound could be sufficient to identify patients in whom surgical approach may be required, without missing clinically significant MTC.

Statement of Ethics

All patients gave their written informed consent, and the study protocol was approved by the institute's committee on human research (Comitato Etico Regione Toscana – Area Vasta Sud Est).

Conflict of Interest Statement

The authors have no conflicts of interest to declare.

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Author Contributions

F.M. and M.G.C. conceived of the presented idea and wrote the manuscript; C.D., N.B., F.P., and M.C. verified the analytical methods. L.B., T.P., M.C., S.C., C.C., and R.F. contributed to the design and implementation of the research; A.C. performed statistical analysis. All authors discussed the results and contributed to the final manuscript.

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