Interpretation of serum calcitonin in patients with chronic autoimmune thyroiditis

Giorgio GRANI¹, Angela NESCA¹, Marianna DEL SORDO¹, Anna CALVANESE¹, Giovanni CARBOTTA¹, Marta BIANCHINI¹, Angela FUMAROLA¹

¹ Dept. of Experimental Medicine, Unit of Endocrinology, “Sapienza” Università di Roma, Rome, Italy

Full postal address for the corresponding author

Prof. Angela Fumarola
Dept. of Experimental Medicine
Sapienza Università di Roma
V.le Regina Elena, 324
00161-Rome
Italy

Phone (+39)-06-49972094

Fax (+39)-06-49972586

Email: angela.fumarola@uniroma1.it

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Abstract

Calcitonin (CT) is an important clinical marker for the diagnosis and follow-up of medullary thyroid carcinoma, although it is not absolutely specific. Some authors have reported C-cell hyperplasia in a number of thyroid specimens affected by Hashimoto’s thyroiditis. The association between thyroiditis and hypercalcitoninemia is still controversial, since some authors have reported low CT levels. The aim of this study is to evaluate basal calcitonin values in patients with and without thyroid autoimmunity.

From May 2005 to February 2010, 1073 patients underwent ultrasonography-guided fine-needle aspiration cytology at the Thyroid Center of Sapienza University of Rome, with evaluation of basal serum FT4, FT3, TSH, anti-thyroid peroxidase (anti-TPO) antibodies, as well as CT levels.

Forty-one patients presented a basal CT level above the reference upper limit. The mean serum CT was significantly lower in women than in men (4.28 ± 6.63 vs 7.50 ± 25.50 pg/mL; p<0.01). Basal serum CT was not significantly higher in patients showing anti-TPO Ab positivity (4.71 ± 6.46 vs 4.84 ± 13.11 pg/mL; p>0.05). Importantly, the rate of “suspicious” CT values (above the 10 pg/mL cut-off) was not significantly different between patients with or without thyroid autoimmunity (3.9% vs 3.0%).

Patients with hypercalcitoninemia suffering from chronic autoimmune thyroiditis should undergo the same clinical evaluation procedure as patients do without thyroid autoimmunity.
**Introduction**

Calcitonin (CT) is a hormone secreted by the parafollicular cells (C cells) of the thyroid gland. The physiologic action of this hormone is uncertain; however, pharmacologically it decreases bone resorption and lowers serum calcium. It is an important clinical marker for the diagnosis and follow-up of medullary thyroid carcinoma (MTC), although it is not absolutely specific. Elevated serum CT levels are usually caused by underlying MTC or C-cell hyperplasia (CCH), a condition defined as the presence of more than 50 C-cells per microscope field (×100) in both thyroid lobes (Scheuba C et al., 2009) and of uncertain biological behavior. Various factors can influence CT secretion, i.e.: physiological (sex, old age, cigarette smoking; Tabassian AR et al., 1989; D’Herbomez M et al., 2007) and pharmacological (consumption of proton-pump inhibitors, glucocorticoids, beta-blockers; Toledo SP et al., 2009). Moreover, several pathological conditions can cause hypercalcitoninemia, e.g.: small-cell lung carcinoma, breast cancer, neuroendocrine tumors, chronic renal failure, pernicious anemia, Zollinger’s syndrome, pancreatitis, hyperparathyroidism, follicular thyroid tumors (Niccoli P et al., 1996), micropapillary thyroid carcinoma (Elisei R, 2008), and sepsis (Becker KL et al., 2010). Some authors have reported CCH in a number of thyroid specimens affected by Hashimoto’s thyroiditis (Guyetant S et al., 1994). However, the association between thyroiditis and hypercalcitoninemia is still controversial (Karanikas G et al., 2004; Schuetz M et al., 2006), since some authors have reported decreased CT levels –in smaller groups of patients– probably caused by atrophy, fibrosis and destruction of both follicular and C-cells (Body JJ et al., 1986; Borges MF et al., 1998; Lima MA et al., 1998; Poppe K et al., 1999). In addition to this, falsely high CT levels (or spurious hypercalcitoninemia) can be caused by the presence of heterophilic antibodies; this effect can be avoided through dilution techniques or sera pretreatment with blocking reagents (Giovanella L & Suriano S, 2011).

**Material and Methods**

From May 2005 to February 2010, 1073 patients underwent ultrasonography-guided fine-needle aspiration cytology (FNAC) at the Thyroid Center of Sapienza University of Rome. The patients were aged 55.70 ± 13.41 (mean ± SD) and all resided in Central Italy, an area of mild to moderate iodine deficiency. Male and female patients were age-matched: 180 men were aged 55.82 ± 13.72, while 893 women were aged 55.67 ± 13.35 (Table 1). Thyroid volume was determined by two-dimensional ultrasonography and calculated by the ellipsoid volume formula with π/6 (0.524) as correction factor. Glands with an estimated volume < 5 ml were
considered atrophic, while thyroid volume > 16 ml was diagnosed as goiter. All patients had at least one
discrete nodular lesion of the thyroid or a multinodular goiter, and were referred to our institution to undergo
FNAC, because of clinical or ultrasonographic suspicion (irregular margins, microcalcifications, chaotic
pattern vascularization).

The current approach at our center is to evaluate basal serum FT4, FT3, TSH, anti-thyroid peroxidase (anti-
TPO) antibodies, as well as CT levels, in all patients in the fasted state. Should suspicious serum CT levels
be detected, the pathologist is thereby asked to perform immunocytochemical staining for calcitonin and
chromogranin. Thyroid functional status was evaluated according to TSH levels: euthyroid between 0.4 and
2.5 mU/l, hypothyroid > 2.5 mU/l and hyperthyroid < 0.4 mU/l. Serum CT was determined using an
automated two-site immunochromiluminometric assay, with functional sensitivity of 2.00 pg/mL and a
reference upper limit of 10.00 pg/mL. Anti-TPO antibodies were measured using RIAs and were considered
positive if above the cutoff point set by the laboratory (>50 U/mL). In patients with positive anti-TPO Ab,
the mean serum TSH value is found to be significantly higher (1.40 vs 1.13 µU/mL; p=0.001) than in patients
with negative antibodies. Cytology results were reported in five categories, as follows, according to the
British Thyroid Association Guidelines and the Thyroid Cytology Italian Consensus SIAPEC-IAP (Fadda G
et al., 2010): (1) non-diagnostic, (2) benign, (3) indeterminate, (4) probably malignant, and (5) positive for
malignant cells.

Statistical analysis

The distribution of CT values was not normal. CT levels were compared using the Mann-Whitney U test
(between two groups) and Kruskal–Wallis test (more than two groups). Categorical variables were compared
using Pearson chi-square test. All tests used a two-sided α of 0.05.

Results

Forty-one patients presented a basal CT level above the reference upper limit. In seven cases, a pathological
cause of hypercalcitoninemia was found: 2 underwent total thyroidectomy with a final histological diagnosis
of medullary thyroid carcinoma (basal CT 141 and 91.1 pg/mL), 2 were chronic renal failure patients
requiring hemodialysis treatment (basal CT 275 and 195 pg/mL), 1 had been diagnosed with MEN but
refused thyroidectomy (basal CT 81.9 pg/mL), and the remaining one patient had a pulmonary carcinoid
tumor (basal CT 52.7 pg/mL). Another patient had thyroid follicular adenoma (basal CT 16.3 pg/mL), but no
CCH was described during histological examination. The other 34 cases remained unexplained, but only 2 patients were found to have persistent hypercalcitoninemia during follow-up (12 and 36 months). These two patients refused to undergo surgery and no other cause was found: atrophic gastritis, hypergastrinemia, hypercalcemia and PPI drugs therapy were ruled out.

Among the 34 cases of idiopathic hypercalcitoninemia, 8 (23.5%) showed thyroid autoimmunity. This rate is not significantly higher than the frequency of autoimmunity in the entire group (19.38%; p>0.05). Ultrasonography showed a goiter with heterogeneous echotexture in 22 cases (64.7%) and an atrophic gland in 2 cases (5.88%).

The overall mean serum CT value was 4.82 ± 12.11 pg/mL. Not including the 7 pathological hypercalcitoninemia patients, the mean value decreased to 4.05 ± 3.49 pg/mL. As expected, the serum CT mean is significantly lower in women than in men (4.28 ± 6.63 vs 7.50 ± 25.50 pg/mL; p<0.01).

This finding is confirmed even if the 7 cases are excluded from evaluation (mean basal CT 3.96 ± 3.42 vs 4.51 ± 3.80 pg/mL; p<0.01). Despite several reports (D’Herbomez M et al., 2007; Toledo SP et al., 2009) that described increased serum CT levels in elderly patients, these data did not display a higher mean value with increasing age. Mean serum CT did not differ according to cytology results, grouped as suggested by British Thyroid Association Guidelines. It should be noted that Thy4 and Thy5 categories indiscriminately include suspicion of papillary, medullary or anaplastic carcinoma, or lymphoma without specification. The rate of “suspicous” CT values (above the 10 pg/mL cut-off) was not different between patients with or without thyroid autoimmunity (3.9% vs 3.0%; p>0.05). Due to the high prevalence of thyroid autoimmunity in females, data were re-analysed including only women to minimize gender-related bias: CT values above the cut-off were recorded in 3.4% of patients with autoimmunity and in 3.1% of patients without anti-TPO Ab positivity (p>0.05). Moreover, basal serum CT was not significantly higher in patients showing anti-TPO Ab positivity (4.71 ± 6.46 vs 4.84 ± 13.11 pg/mL; p>0.05). These observations remain valid when the 7 pathological cases of high CT levels are excluded (4.34 ± 3.59 vs 3.98 ± 3.46 pg/mL; p>0.05) and when only women are considered (4.00 ± 3.12 vs 3.95 ± 3.49 pg/mL; p>0.05).

No difference was recorded in mean serum CT between patients with goiter or with atrophic gland, respectively (3.84 ± 3.49 vs 5.10 ± 5.20 pg/mL; p>0.05). Results are summarized in Table 2.

Discussion
Routine serum calcitonin screening is useful in patients undergoing thyroid nodule evaluation, particularly in the presence of suspicious cytological findings (Elisei R et al., 2004; Papi et al., 2006; Costante et al., 2007), and appears to be cost-effective (Cheung K et al., 2008). CT levels >100 pg/mL are widely considered an indication for surgery (Kloos RT et al., 2009). The management of patients showing a slight increase in CT levels (a condition that is relatively more frequent) appears to be more controversial. In the present study, the prevalence of MTC is only 0.19%, while basal serum CT levels above the reference range were recorded in 3.82% of cases. A number of causes of spurious hypercalcitoninemia can be ruled out by obtaining a careful clinical history. Male gender and re-evaluation with dilution techniques or sera pretreatment in blocking tubes must be considered when evaluating borderline values. Nevertheless, Hashimoto's thyroiditis does not influence basal CT levels, when measured with a sensitive two-site ILMA assay. Patients with hypercalcitoninemia suffering from chronic autoimmune thyroiditis should undergo the same clinical evaluation procedure as do patients without thyroid autoimmunity.

Declaration of interest

There is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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References


Toledo SP, Lourenço DM Jr, Santos MA, Tavares MR, Toledo RA & Correia-Deur JE 2009

Hypercalcitoninemia is not pathognomonic of medullary thyroid carcinoma. *Clinics (Sao Paulo)* 64 699-706.
Mean age ± SD (yr) 55.70 ± 13.41
Males (n=180), mean age ± SD (yr) 55.82 ± 13.72
Females (n=893), mean age ± SD (yr) 55.67 ± 13.35

Thyroid function

Euthyroidism 809 (75.40%)
(Subclinical) Hypothyroidism 78 (7.27%)
(Subclinical) Hyperthyroidism 186 (17.33%)

Thyroid autoimmunity

Anti-TPO antibodies + 208 (19.38%)
Anti-TPO antibodies - 865 (80.62%)

Basal serum CT

Under the cut-off 1032 (96.18%)
mean age ± SD (yr) 55.72 ± 13.42
Above the cut-off 41 (3.82%)
mean age ± SD (yr) 55.17 ± 13.20
Of which:
Pathologic hypercalcitonemia 7 (17.07%)
Idiopathic hypercalcitonemia 34 (82.93%)

Table 1. Age and thyroid status data.
<table>
<thead>
<tr>
<th></th>
<th>All patients (n=1073)</th>
<th>Patients without recognized pathologic increase of serum CT (n=1066)</th>
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<tbody>
<tr>
<td></td>
<td>Basal serum CT (pg/mL), mean ± SD</td>
<td>p</td>
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<tr>
<td><strong>Sex</strong></td>
<td></td>
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</tr>
<tr>
<td>Male</td>
<td>7.50±25.50</td>
<td>&lt;0.01*</td>
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<tr>
<td>Female</td>
<td>4.28±6.63</td>
<td></td>
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<tr>
<td><strong>Age</strong></td>
<td></td>
<td></td>
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<tr>
<td>&lt; 65 yr</td>
<td>4.37 ± 6.71</td>
<td>&gt;0.05*</td>
</tr>
<tr>
<td>≥ 65 yr</td>
<td>5.96 ± 20.09</td>
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<tr>
<td><strong>TPO</strong></td>
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<tr>
<td>Negative</td>
<td>4.84 ± 13.11</td>
<td>&gt;0.05*</td>
</tr>
<tr>
<td>Positive</td>
<td>4.71 ± 6.46</td>
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<tr>
<td><strong>Citology</strong></td>
<td></td>
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<tr>
<td>Thy1</td>
<td>4.86 ± 14.64</td>
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<tr>
<td>Thy2</td>
<td>4.57 ± 8.95</td>
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<tr>
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<td>Thy4</td>
<td>3.65 ± 2.38</td>
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<tr>
<td>Thy5</td>
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Table 2. Basal serum calcitonin (pg/mL, mean ± SD) in sub-groups of patients. * Mann-Whitney U test □ Kruskal Wallis test