Case Report

# False positive results using calcitonin as a screening method for medullary thyroid carcinoma

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## ABSTRACT

The role of serum calcitonin as part of the evaluation of thyroid nodules has been widely discussed in literature. However there still is no consensus of measurement of calcitonin in the initial evaluation of a patient with thyroid nodule. Problems concerning cost-benefit, lab methods, false positive and low prevalence of medullary thyroid carcinoma (MTC) are factors that limit this approach. We have illustrated two cases where serum calcitonin was used in the evaluation of thyroid nodule and rates proved to be high. A stimulation test was performed, using calcium as secretagogue, and calcitonin hyper-stimulation was confirmed, but anatomopathologic examination did not evidence medullar neoplasia. Anatomopathologic diagnosis detected Hashimoto thyroiditis in one case and adenomatous goiter plus an occult papillary thyroid carcinoma in the other one. Recommendation for routine use of serum calcitonin in the initial diagnostic evaluation of a thyroid nodule, followed by a confirming stimulation test if basal serum calcitonin is showed to be high, is the most currently recommended approach, but questions concerning cost-benefit and possibility of diagnosis error make the validity of this recommendation discussible.

**Key words:** Calcitonin, carcinoma, medullary, thyroid diseases

## Introduction

Medullary thyroid carcinoma (MTC) is a neoplasia of parafollicular cells or of thyroid C-cells, which represents approximately 4% of thyroid malignant tumors. Approximately 75% are sporadic and 25% are inherited.[1] They can be part of Multiple Endocrine Neoplasia (MEN) type 2, 2A or 2B, or occur as a Familial MTC or other inherited forms. Molecular studies to identify genetic mutation responsible for MTC started in 1970 and, in 1993 and RET proto-oncogene was identified as the responsible for occurrence of this neoplasia.[2-4] Among various substances produced by C cells the most important one is calcitonin that is used for detection, post-operative follow-up and evaluation of individuals at risk of developing MTC. Prognosis of MTC is related to the cure of the disease at the moment of the diagnosis, and this disease is usually diagnosed when there is already a metastatic involvement.[5] It is estimated that 0.4 to 1.37% of patients with thyroid nodules have MTC.[6,7]

As an attempt to increase the number of diagnoses at an earlier stage, in 1994, the routine use of calcitonin in the evaluation of thyroid nodules was proposed.[8] Since then the role of serum calcitonin in the evaluation of thyroid nodules has been widely discussed and there is still no consensus about the administration of blood calcitonin measurement in the initial evaluation of all thyroid nodules. Problems related to: Cost-benefit, lab evaluation methods, false positives and low prevalence of medullary thyroid carcinoma are supported by authors who consider it unnecessary to include calcitonin in the evaluation of a thyroid nodule.[9,10] A higher expectation of cure for a neoplasia that usually has a late diagnosis, and whose prognosis can be altered by its early detection, is the main reason that justifies serum calcitonin levels measurement as a screening test for MTC in all thyroid solid or cystic-solid nodules.[6,7,11]

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Studies that have analyzed the role of calcitonin measurement in the investigation of thyroid nodules applied a strategy that has used levels of basal calcitonin followed by a stimulation test for calcitonin (usually pentagastrin) in cases with high serum calcitonin levels, for a more specific confirmation.[4-8]

Application of these procedures, however, is not exempt from error. High calcitonin levels can be detected in other thyroid and extra-thyroid pathologies, neoplastic or not.[9] The stimulation test seems to provide a more specific diagnosis, but false positive results may also occur.

## Case Reports

### Case 1

A 32-year-old, female, white, Brazilian patient has sought medical advice with an endocrinologist as she noticed a lump in the cervical region. Thyroid palpation indicated an enlargement of the gland of about three to four times with irregularities in the lower left third and a palpable nodule in the lower right third. Ultrasound evidenced two nodules in the left lobe (0.6 × 0.9 and 1.0 × 1.0 cm), the lower third of the last one demonstrated hyperechoic and hypovascularized region. A solid nodule was also found next to the lower pole of the left lobe with no continuity to it, of 1cm the diameter. Normal levels of serum TSH and free T4, total and ionic serum calcium, inorganic phosphorus and chlorine were showed, and anti-thyroglobulin and anti-thyroperoxidase antibodies were reactants.

Calcitonemia was 10 pg/mL (normal levels=<2 pg/mL). Thyroid nodule fine needle aspiration was done under direct visualization with ultrasound, and anatomopathologic examination disclosed a follicular pattern. The immunohistochemical staining of the aspirated material was negative for calcitonin, diffusely positive for thyroglobulin, negative for chromogranin and carcinoembryonic antigen, positive for AC1 and AC3, negative for galectin-1, negative for HBME-1, and positive for TTF-1. Stimulation test for calcitonin was performed and response to the test was positive, reaching a maximum of 79 pg/mL 2 minutes after calcium infusion [Table 1]. The patient underwent a thyroidectomy due to the follicular lesion. The congealing examination evidenced chronic lymphocytic thyroiditis in the nodules. Immunohistochemical staining was positive for calcitonin in the inflammatory and in the C cells with no evidence of C cells hyperplasia. Post-operative hypothyroidism was appropriately treated with levothyroxine. Serum levels of thyroglobulin and calcitonin levels were undetectable in the post-operative evaluation.

**Table 1: Dosage of thyroglobulin, calcitonin and TSH after infusion of recombined TSH in times 0, 48, and 96 hours, respectively**

| Hours | 0 | 48 | 96 |
|---|---|---|---|
| Thyroglobulin | Undetectable | 1 | 1 |
| Calcitonin | <2 | <2 | <2 |
| TSH | 38.7 | 68 | 40.7 |

TSH: Thyroid-stimulating hormone

### Case 2

A 59-year-old, male, Egyptian patient, started treatment for obesity with an endocrinologist, taking sibutramine and orlistat. After clinical examination a thyroid ultrasound was performed and evidenced hypoechogenic solid nodule of 1.2 cm the diameter in the upper third of the right lobe and an adjacent cyst with parietal calcifications of 0.6 cm. He had normal TSH and free T4, with negative anti-thyroperoxidase and anti-thyroglobulin antibodies. Serum basal calcitonin levels of 19 pg/mL were detected (normal levels=<2 pg/mL). Stimulation test for calcitonin was performed reaching a rate of up to 890 pg/mL, observed at two minutes after calcium infusion [Table 2]. Total thyroidectomy was performed. During surgery four nodules were identified in the right lobe and two nodules in the left lobe. In the anatomopathologic examination 12 nodules were observed, evidencing adenomatous goiter, except one white, calcified and encapsulated 0.3 cm nodule classified as a classic variable papillary microcarcinoma. Among the nodules, in both lobes, diffuse hyperplasia of C cells was observed [Figure 1], however with no presence of atypia. Immunohistochemistry was positive for chromogranin and calcitonin in numerous interstitial and intrafollicular cells [Figure 2]. Three months after surgery, measurement of thyroglobulin and calcitonin levels was performed, after two injections of recombined TSH, and rates for both of them were undetectable what remain for five years [Table 2].

## Discussion

MTC is a malignant neoplasia originated from the parafollicular C-cells of the thyroid gland. It has a more aggressive behavior than the well-differentiated thyroid carcinoma and a 40-50% 10-year survival rate. Seventy-five per cent of medullar carcinoma are sporadic and the remaining 25% are part of the familial medullar carcinoma (FMC) or of the multiple endocrine neoplasia type 2 (MEN-2).[7] Among all thyroid neoplasias, MTC corresponds to approximately 4%.[8] When medullar carcinoma is detected in the form of a thyroid nodule, it usually has already developed metastases.[5] Therefore, early diagnosis is fundamental to improve survival rates of this neoplasia. With that in mind, the use of calcitonin has been suggested in routine evaluation of a thyroid nodule, what

**Table 2: Biochemical and immunological measurements before and after surgery**

| | Case 1 | | Case 2 | | Reference rates |
|---|---|---|---|---|---|
| | Pre-op. | Post-op. | Pre-op. | Post-op. | |
| Biochemical dosages | | | | | |
| Calcitonin (pg/mL) | 10 | <2 | 19 | <2 | <2 |
| TSH (mUI/L) | 1.4 | 4.3 | 1.6 | 3.6 | 0.45-4.5 |
| FreeT4 (ng/dL) | 0.9 | 1.1 | 1.0 | 1.1 | 0.7-1.5 |
| Calcium (mg/dL) | 9.8 | 7.7 | 10.0 | 9.8 | 8.4-10.2 |
| Ionic calcium (mg/dL) | 1.25 | 1.1 | 1.2 | 1.0 | 1.14-1.31 |
| Phosphorus (mg/dL) | 3.5 | 4.2 | 3.7 | 3.7 | 2.3-4.3 |
| Chlorine (mEq/L) | 103 | - | 103 | - | 100-108 |
| Immunological dosages | | | | | |
| Anti-thyroglobulin antibody | Reactive | 130 | Negative | Negative | Negative |
| Anti-thyroperoxidase antibody | Reactive | 7900 | Negative | Negative | Negative |
| Stimulation testing using calcium (pg/mL) | | | | | |
| 0 minutes | 6 | - | 22 | - | - |
| 2 minutes | 79 | - | 890 | - | - |
| 5 minutes | 63 | - | 600 | - | - |
| 10 minutes | 31 | - | 390 | - | - |
| 15 minutes | 42 | - | 280 | - | - |

TSH: Thyroid-stimulating hormone

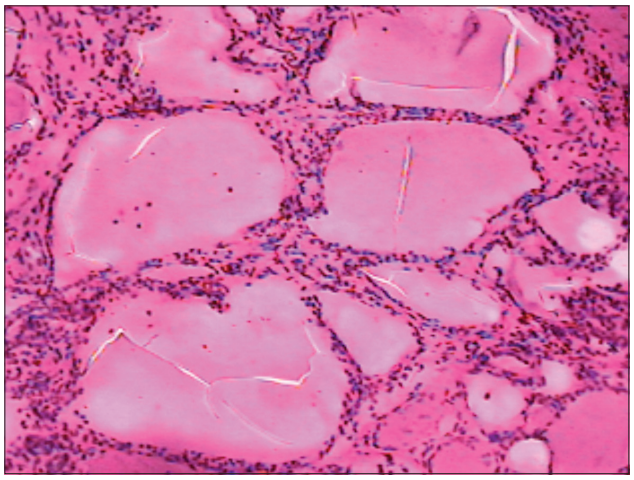

**Figure 1:** Case 2: Thyroid parenchyma showed follicles preserved, with foci of increased stromal represented by isolated cells with ample cytoplasm finely granular (C cells hyperplasia)

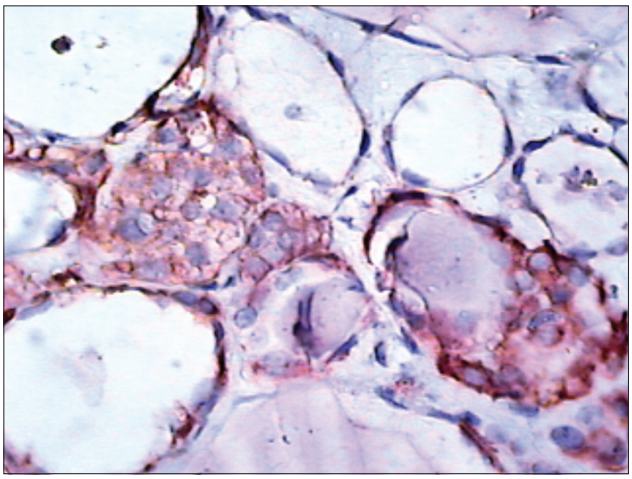

**Figure 2:** Case 2: C cells showing positive chromogranin, but without the presence of atypical cells

allows identification of a greater number of early diagnosed patients and, therefore, reduce morbidity and mortality.[4-8]

In 1994, Pacing *et al.,* published an article suggesting that serum calcitonin should be used in the initial diagnostic evaluation of every thyroid nodule, because diagnosis of MTC is frequently post-operative as fine needle aspiration (FNA) fails to diagnose the disease and the diagnosis of MTC at an earlier stage could modify the prognosis of this neoplasia.[5] FNA in the diagnosis of medullar neoplasia has a limited value.[5,10,12] A study that has compared FNA with anatomopathologic results evidenced that FNA could identify only two among eight cases with anatomopathologic diagnosis of medullar carcinoma.[12] Many FNA are interpreted as benign in those situations. In general, FNA can diagnose, in the preoperative stage, about less than half of the cases of medullary thyroid neoplasia.[10] Comparing serum calcitonin with FNA, calcitonin proved to be high in 100% of the cases of medullar carcinoma while FNA could identify just 22.2% of those cases.[5]

Saller *et al.,* have studied the role of Doppler and conventional ultrasound in the diagnosis of MTC and observed that conventional ultrasound revealed a combination of hypoechogenicity, intranodular calcifications and absence of halo in 89% of the patients with medullar carcinoma, in contrast to 6% in cases of benign thyroid nodules, what suggests the use of this tool as a screening method for medullar neoplasia.[13] In the two cases reported here, that association could not be found.

Recommendation for the use of calcitonin followed by a stimulation test after identification of basal hypercalcitonemia

may not be easy to be performed as pentagastrin is not available in some countries.[14,15] To provide us with an estimate of the costs, in France a serum calcitonin dosage costs about €24. Thus, routine screening for all thyroid nodule patients would cost €18 million. Furthermore, up to the moment, a rate of basal calcitonin that can separate benign from malignant diseases has not been all established.[16] A variability of lab methods used to evaluate calcitonin turns it into a knotty problem, and also makes it more difficult to compare data from different studies.[17]

However, most of the studies that analyze calcitonin as a screening method for MTC support this approach as the only possibility of an early MTC preoperative diagnosis, due to inefficiency of FNA and suggest that basal calcitonin should be performed in all cases of thyroid nodular disease and that, in cases of high rates, a stimulation test using calcium and/or pentagastrin should also be performed.[18,19] Vierhapper *et al.,* have measured basal calcitonin in 14,000 patients, 10,158 of them with thyroid nodules. Among patients with thyroid nodules, 507 indicated basal calcitonin higher than 10 pg/mL. In all these patients a stimulation test using pentagastrin (0.5 μg/kg EV) was performed and 103 indicated calcitonin higher than 100 pg/mL after stimulation. In this group, 32 new cases of MTC were identified and 43 patients with HCC. It was estimated that, after instituting routine use of calcitonin in the nodular evaluation, the number of diagnosis of MTC increased from 1.1 cases in 1,000 patients to 3.2 cases in 1,000 patients. On the other hand both cases reported in this study illustrate possibilities of false positives that may occur in high basal serum calcitonin levels what reinforced surgical indication. Evidence of a high level of calcitonin in the initial approach led to a performance of a stimulation test where an increase in serum calcitonin occurred. None of the cases were a MTC.

False positive increases in calcitonin may occur in situations like benign hyperplasia of C cells, benign nodules, differentiated thyroid carcinoma and Hashimoto thyroiditis (HT).[14,15] No thyroid factors may also cause serum calcitonin to increase like: Neuroendocrine tumors, sepsis, older age, generalized inflammation and physical exercise.[11-13]

In Case 1, there was a slight increase in serum calcitonin in two evaluations using immunoradiometric assay from kits available. That led to a stimulation test using calcium, indicating high peak rates (>40 pg/mL). As FNA evidenced follicular lesion, a surgery was performed. As previously reported, the anatomopathologic diagnosis was Hashimoto thyroiditis.

Recently, Karanikas *et al.,* have reported a prevalence of 25% of increase in serum basal calcitonin levels in patients with Hashimoto thyroiditis, indicating a significant relation between nonnodular thyroid disease and high levels of serum calcitonin.[14] This author proposed measurement of calcitonin not only in patients with thyroid nodules but also in patients with HT, what we do not agree. Irrespective of the results from the discussion this proposal may cause, the occurrence of HT is a relevant factor to hypercalcitonemia by not all established mechanisms, and may lead to false positive results when measurement of calcitonin, basal or stimulated, is performed as a screening method. One hypothesis to hypercalcitonemia is that C cells response may be neoplastic or physiological. In HT, as well as in other situations related to the increment in serum calcitonin levels, either a physiological or a reactive response seems to occur, different from the response observed in HCC related to MTC.[14] In these situations, basal calcitonin may be high, but rates after stimulation are usually lower than the ones observed in cases of MTC.[14,20]

In the second case, the patient also indicated high serum calcitonin levels, in two evaluations, using immunoradiometric assay from kits available. Stimulation test using calcium evidenced high rates, suggesting medullar neoplasia (890 pg/mL). However, anatomopathologic diagnosis evidenced adenomatous goiter in eleven nodules, papillary thyroid microcarcinoma in one nodule and diffuse C cells hyperplasia (CCH). We believe, in this case, that this huge increment is a result of C cells hyperplasia. HCC is a known reason for increases in levels of stimulated and basal calcitonin, what frequently makes it difficult to differentiate this situation from cases like medullar microcarcinoma and MTC itself.[21] Gibelin[22] considers that in cases when preoperative rates of stimulated calcitonin range between 30 and 100 pg/mL it is difficult to predict the presence of HCC or of medullar microcarcinoma, but he considers that rates higher than 100 pg/ml are strong evidences of MTC. Differentiated thyroid tumors may cause increase in serum calcitonin, similar to the one observed in nonneoplastic diseases therefore much lower than that evidenced in MTC cases.[20] The main concern here is the rate of increase in calcitonin after the stimulation test. There is no established rate that can biochemically differentiate C cells hyperplasia from MTC. As an attempt to answer this question and to determine a biochemical rate that could differentiate C cells hyperplasia from MTC, Scheuba *et al.,* demonstrated levels of basal calcitonin higher than 64 pg/mL or a rate of stimulated calcitonin higher than 560 pg/mL in 31 of 38 patients that underwent a thyroidectomy with histological diagnosis of medullar carcinoma, assuming that these rates are a prediction of medullar carcinoma in 81% of the cases. They also observed that patients with stimulated calcitonin lower than 129 pg/mL had only hyperplasia of C cells and not medullar carcinoma. Among

patients with medullar carcinoma, the ones who indicated basal calcitonin lower than 22 pg/mL did not have positive lymph nodes.[15] Iacobone *et al.,* proposed, in 2002, cut-off rates for basal and after stimulation calcitonin, indicating that basal rates higher than 30 pg/ml and post-stimulation rates higher than 200 pg/ml have 61.3% sensitivity, 100% specificity and 100% positive prediction value.[23] In that study, cases diagnosed as MTC, rates of basal calcitonin ranged between 145 and 205 pg/mL.

## Conclusions

An effective screening method may be able to modify the prognosis of MTC. Routine use of serum calcitonin followed by a confirming stimulation test in the cases of high basal levels is the most recommended approach, due to inefficiency of FNA and lack of better data on the role of ultrasound for MTC. Questions concerning cost-benefit and the possibility of false positives may limit this recommendation, but it is clear that this approach increases the number of diagnosis of MTC. Although there still are many questions regarding administration of calcitonin, this is a valid, factual and relatively simple method that allows the preoperative diagnosis of MTC. One should be careful when there is evidence of a condition different from MTC that is known to increase calcitonin or when there is a slight increase in the levels of calcitonin, basal or after stimulation. Subsequent studies should be carried out to establish appropriate rates that could separate benign from malignant diseases, in order to reduce possible errors.

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