

Calcitonin stimulation tests for the early diagnosis and follow-up of patients with C cell disease: a descriptive analysis

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Abstract

Background/Aim: Residual or recurrent medullary thyroid carcinoma (MTC) after thyroidectomy is diagnosed by elevated serum calcitonin (CT) levels. However, in minimal residual MTC or C-cell hyperplasia (CCH), where imaging studies are often negative, basal CT levels are frequently normal and CT stimulation tests are required. We aimed to compare CT stimulation tests with calcium, pentagastrin and their combination in identifying minimal residual MTC and CCH.

Material and methods: We studied 10 post-thyroidectomy patients with MTC and 20 first-degree relatives of the patients who had no clinically apparent MTC. We performed 54 combined (calcium plus pentagastrin) stimulation tests, 35 calcium stimulation tests and 26 pentagastrin stimulation tests.

Results: Basal CT levels were abnormal (≥ 500 pg/ml) in 4 patients with apparent metastatic disease (Group 1A) and in 2 patients with minimal residual disease (Group 1B) but were normal (0-300 pg/ml) in 4 patients with no residual disease (Group 1C) and in the relatives (Group 2). In Groups 1A, 1B and 1C, maximal elevation in CT levels was greater after the combined stimulation test than after calcium or pentagastrin tests. The combined stimulation test induced the greatest increases (920, 700 and 706 pg/ml, respectively) in 3 relatives (Group 2); CCH was confirmed histologically in these patients. Side-effects were mild, short-lasting and of similar intensity and duration during all tests.

Conclusions: Patients with subclinical MTC (minimal residual or recurrent MTC) or their relatives (with CCH) usually have normal basal CT levels and stimulation tests are necessary. Combined test represents the most sensitive and safe stimulation test for the diagnosis of subclinical hypercalcitonemia. Hippokratia 2013; 17 (3): 246-251

Keywords: Medullary thyroid carcinoma, C cell hyperplasia, calcium stimulation test, pentagastrin stimulation test, combined stimulation test, residual disease

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Introduction

Medullary thyroid carcinoma (MTC), a malignant neoplasm of parafollicular C cells¹, is characterized by excessive presence of immunoreactive serum calcitonin (CT). More specifically, some patients have elevated basal CT levels whereas others show an abnormal increase in CT levels only during stimulation tests with various agents, including calcium gluconate (Ca²⁺) and pentagastrin (Pg), which stimulate CT secretion from MTC or C-cell hyperplasia (CCH). Patients with clinically apparent MTC and elevated basal serum CT concentrations do not require stimulation tests to confirm the diagnosis of MTC^{2,3}.

However, basal serum CT levels are normal in 30% of patients with hereditary MTC with microscopic neck lymph node metastases and in 50% of patients with sporadic MTC who had undergone thyroidectomy and ex-

cision of involved neck lymph nodes⁴ but are not cured after surgery and have minimal residual neck disease. In these patients, stimulation tests with Ca²⁺ or Pg induce a rise in CT levels and represent well established diagnostic tests which also have prognostic value⁵⁻⁹. Other agents that stimulate CT secretion from normal or neoplastic C cells include thyrotropin releasing hormone¹⁰, omeprazole^{11,12} and thyrocalcitonin¹³. In patients with minimal disease, imaging studies including ultrasound¹⁴, magnetic resonance imaging (MRI)¹⁵, scintigraphy¹⁶ and even ¹⁸F-FDG-positron emission tomography (PET)¹⁷ often fail to identify micrometastases, which are usually multiple with multiple foci in each involved organ. Thus, measuring serum CT levels after a stimulation test could detect a MTC at an early stage and identify the few patients with aggressive disease who might be candidates for new treatment modalities. In the latter patients, moni-

toring of CT levels might also be useful not only in the assessment of the treatment response, but serum CT and carcinoembryonic antigen (CEA) levels are also sensitive markers of tumor burden and dissemination.

Basal serum CT levels are also within the normal range in >90% of the first-degree relatives of patients with familial MTC and stimulation tests can help identify sub-clinical CCH (a pre-cancerous disease) in these subjects; the combined administration of Ca^{+2} and Pg has been suggested to be more sensitive in the latter subjects⁹.

We aimed to assess the role of serum CT stimulation tests (with Ca^{+2} , Pg or their combination) in the identification of occult metastases in patients who had undergone surgery for MTC and in the early diagnosis of CCH in their relatives. We also recorded the type, severity and duration of side-effects during each stimulation test.

Material and Methods

Subjects and methods

We studied 10 patients who had undergone surgery for MTC and 20 first-degree relatives of theirs [18/30 females (60%), median age 35 years (range 18-52 years)]. Two of the patients and none of their relatives had multiple endocrine neoplasia (MEN) type 2A. Among the 10 patients with MTC, 4 patients had clinically apparent metastatic disease (cervical lymphadenopathy or pulmonary, liver or bone metastases) despite multiple operations and alternative antineoplastic treatments and died after 1, 3, 4.5 and 6 years of follow-up, respectively (Group 1A) (Table 1).

Two patients had initially minimal residual disease (very few and small, hardly palpable metastatic cervical lymph-nodes; Group 1B) that recurred after 2 operations and progressed slowly after 12 and 16 years of follow up. Finally, 4 patients had no residual disease (Group 1C) clinically or on usual imaging methods and did not relapse after a follow-up of 9, 11, 12 and 17 years, respectively. All patients had sporadic MTC except 1 patient from Group 1A and her son from Group 1B, who had MEN type 2A.

Patients older than 60 years were excluded from the study whereas younger patients with coronary heart disease, migraine, peptic ulcer or other systemic diseases underwent only the Ca^{+2} stimulation test.

We also studied 20 first-degree relatives of the 10 patients with MTC (Group 2) (Table 1). These 20 subjects had no clinical or imaging evidence of MTC or C-cell-hyperplasia and had not previously undergone screening for the presence of the RET proto-oncogene. Four of these 20 subjects are relatives of the 2 patients with the MEN 2A syndrome.

All patients and all relatives provided written informed consent before undergoing any stimulation test.

Measurement of serum CT levels

Serum CT was determined with a radioimmunoassay (RIA-mat Calcitonin I, Mallinckrodt Diagnostica, 6057 Dietzenbach 2, Germany) with sensitivity < 0.078 pg/ml. Non-specific binding with this assay was < 5%, was determined during all stimulation tests and was accounted for when measuring CT levels. Intra-assay coefficient of variation (CV) was 11.3%, 4.9% and 5.8% at CT levels of 210, 670 and 1130 pg/ml, respectively. Mean intra-assay CV was 8.3% at CT levels of 1090 pg/ml.

All data were analyzed using the statistical package SPSS (version 13.0; SPSS Inc., Chicago, IL, USA) and are presented as medians and range.

Clinical protocol

The presence of clinically apparent or minimal residual MTC was assessed with clinical examination, ultrasonography, computed tomography and MRI (and PET in selected patients) and was confirmed with post-operative pathological examination. Metastases included multiple cervical lymph nodes bilaterally and/or nodular lung metastases who were identified during selective or modified lymph node dissection or with imaging studies.

Serum CT levels were measured at baseline and after stimulation tests with either a) Ca^{+2} 2 mg/kg infused i.v. within 50 sec b) Pg (Peptavlon, Ayerst) 0.5 $\mu\text{g}/\text{kg}$ infused i.v. within 10 sec or c) Ca^{+2} 2 mg/kg (50 sec i.v. infusion) followed immediately by Pg 0.5 $\mu\text{g}/\text{kg}$ (10 sec i.v. infusion) (combined stimulation test). Serum CT levels were determined immediately after the end of the infusion of the stimulating agents (time 0) and 1, 2, 3.5, 5 and 7 min later.

The combined stimulation test was performed in 25 of the 30 subjects and all 3 stimulation tests were performed in 14 of the 30 subjects.

Table 1: Groups of patients included in the study.

Group	n	Characteristics
1A	4	Patients with clinically apparent metastatic MTC
1B	2 10	Patients with minimal residual disease
1C	4	Patients without residual disease
2	20	First-degree relatives of patients with MTC without clinical or imaging abnormalities

MTC: medullary thyroid carcinoma.

CT levels >500 pg/ml (i.e. ~2 times higher than the upper limit of the normal range of serum CT levels) suggested the presence of residual or recurrent MTC. In contrast, CT levels between 300-500 pg/ml were considered suspicious and these patients were retested after 6 to 12 months.

Stimulation tests were also performed in the 4 patients of Group 1A and in the patient of Group 1B who had elevated basal CT levels to compare the increase in CT levels during the 3 different stimulation tests.

Patients with MTC underwent a second surgery and patients in groups 1A, 1B or even in group 1C (if recurrent or microscopic residual disease was detected) underwent total thyroidectomy and lymphnode dissection if a) fine-needle aspiration biopsy suggested the presence of MTC, b) they had operable metastatic disease, c) a suspicious ultrasound imaging and d) basal or post-stimulation CT levels were > 500 pg/ml.

The 20 first degree-relatives were assessed with stimulation tests because for technical reasons it was not initially possible to perform genetic analysis for the presence of mutations of the RET oncogene. Moreover, 4 of these subjects had to be assessed with stimulation tests because they were relatives of patients with the MEN 2A syndrome. Analysis for the presence of mutations of the RET oncogene was performed in all 10 patients with MTC 1-2 years after the first stimulation test and identified a mutation in 2 of them (the 2 patients with MEN 2a syndrome).

During the follow-up of the 20 first-degree relatives (range 1.5-17 years), the following tests were performed: i) physical examination, ii) measurement of basal CT levels, iii) thyroid and neck ultrasound, iv) genetic analysis for the presence of mutations of the RET oncogene in 15 of them that identified a mutation in only 3 of them.

Side effects were recorded by 2 experienced physicians using a questionnaire that recorded the type of side effect. During the stimulation test, patients were also monitored by an experienced nurse. The severity of the side effects was recorded using a semi-quantitative scale as mild (not bothersome for the patient), moderate (bothersome but tolerable) and severe (intolerable). Severe side-effects were short-lasting and did not mandate the discontinuation of the stimulation test or any specific treatment. The duration of the side effects was recorded between the end of the infusion of the stimulatory agents (time 0) and 0.5, 1, 2, 3 and 5 min.

Pathologic confirmation

The diagnosis of MTC was based on sensitive and specific pathological features, including

a) well demarcated, whitish and firm nodules, consisting of sheets or nests of uniform, small, round, polygonal or spindle-shaped cells, with round or spindled medium-size nuclei, without nucleoli, with azurophilic cytoplasmic granules and with amyloid deposits in ~80% of the cases.

b) cells separated by variable amounts of fibrous stroma, with variable amount of collagen.

c) positive immunochemistry for calcitonin, chromogranin-A, CGRP, and sometimes for CEA or for epithelial markers, and negative immunochemistry for thyroglobulin in at least 3 optical fields containing at least 50 C-cells.

The diagnosis of CCH was based on classical histological characteristics, including

a) minute populations of C-cells, multifocal and in both thyroid lobes

b) diffuse hyperplasia of the C-cells, which were more abundant and larger

c) large, hyperdense, round or spindled C-cells with numerous granules, located inside the thyroid follicles but clearly demarcated by the interstitial space and the basal membrane

d) positive immunochemistry for CT and low-molecular weight keratins and negative immunochemistry for CEA, CGRP and thyroglobulin.

Results

All 4 patients of group 1A had abnormal basal serum CT levels (between 817 and 10,600 pg/ml) and peak levels ranged between 3,900-9,770 pg/ml, 12,000-13,770 pg/ml and 11,210-37,000 pg/ml during the Ca⁺², Pg and combined stimulation tests, respectively. Peak serum CT levels were recorded within 0-3.5 min after the combined test.

Regarding the 2 patients of group 1B, one had basal serum CT levels normal-suspicious (0-411 pg/ml) and the other abnormal (731-3,650 pg/ml). Serum CT concentration reached abnormal levels in both patients 2 and 5 minutes after the combined stimulation test (1,970-7,749 and 1,530-5,200 pg/ml, respectively) or the Pg stimulation test (1,930-12,800 and 8,190 pg/ml, respectively), but not with the Ca⁺² stimulation test (167 pg/ml) in the former patient.

All 4 patients from group 1C had normal basal serum CT levels following the 3 stimulation tests (range 0-100 pg/ml). Peak CT levels following the 3 stimulation tests did not exceed 420 pg/ml in any patient. These suspicious levels were observed in 1 patient after the combined and the Pg stimulation test (350 and 420 pg/ml), but not after the Ca⁺² stimulation test. However, the former patient did not relapse after a follow up of 17 years.

All subjects in Group 2 had normal basal serum CT levels (median 50 pg/ml, range 0-220 pg/ml). Three subjects from this group showed an abnormal increase in CT levels (i.e. peak levels of 920, 700 and 720 pg/ml, respectively) after the combined stimulation test. One of these 3 patients underwent all 3 stimulation tests, the second underwent the combined and Ca⁺² test whereas the third patient underwent only the combined test; none showed an abnormal increase in CT levels during the isolated Ca⁺² or Pg stimulation tests. All these 3 patients underwent total thyroidectomy and the presence of CCH was confirmed at the pathologic evaluation. These 3 patients were relatives of the 2 patients with familial MTC (MEN 2A) and probably suffer from familial C-cell disease. The other

17 subjects of group 2 did not show abnormal increases in CT levels during any stimulation test. Except the 3 subjects of this Group mentioned above, no other subject in this Group developed CCH or MTC during follow-up (range 1.5-17 years); no subject in this Group was lost to follow-up.

The type and absolute number of side effects are shown in Table 2. A burning sensation in the throat and genitals was the commonest side-effect in patients with MTC during the 3 stimulation tests. Weakness and abdominal discomfort were also reported by Group 2 (plus face flushing after Pg), but not in patients with MTC. One patient experienced angina during the Pg stimulation test. The symptoms lasted 2 min and resolved without any intervention or change in electrocardiogram or in myocardial enzyme levels. Patients undergoing the combined

test reported a combination of these side effects as well as flushing. The majority of side effects were of mild-to-moderate intensity (76, 77 and 61% of all side effects after the combined, Ca⁺² and Pg stimulation tests, respectively; Table 3). The duration of side effects was 0.5-1 min in 59%, 29.4% and 20% of all side effects after the combined, Ca⁺² or Pg stimulation tests, respectively (Table 4).

Discussion

Metastases to the cervical lymph-nodes occur early in the course of MTC and approximately 50% of these patients have metastatic cervical lymphadenopathy at the time of first diagnosis¹⁸, but cervical ultrasonography or guided fine-needle aspiration performed before surgery frequently fails to identify these metastases¹⁹. In addition,

Table 2: Type and absolute number of side effects in patients and relatives during each stimulation test.

	Calcium test		Pentagastrin test		Combined test	
	Patients (n=11)	Relatives (n=14)	Patients (n=7)	Relatives (n=8)	Patients (n=10)	Relatives (n=16)
Flushing	0	1	0	0	1	8
Faintness	0	1	1	0	0	0
Nausea	3	3	2	2	2	2
Weakness	1	2	0	5	2	3
Malaise	1	1	0	0	1	0
Numbness	2	2	0	1	0	2
Abdominal discomfort	1	0	1	4	0	5
Angina/palpitations	0	1	1	1	0	0
Burning sensation	10	9	5	2	10	15
Tachycardia	0	0	0	1	0	2
Dysarthria	0	0	0	0	0	1
Hunger	0	0	0	1	0	2
No side-effects	0	1	0	2	1	0

Table 3: Severity of side-effects recorded during all the stimulation tests in the 10 patients with medullary thyroid carcinoma and in their 20 first-degree relatives.

Stimulation test	Without side-effects		Mild		Severity Moderate		Severe	
	Relatives	Patients	Relatives	Patients	Relatives	Patients	Relatives	Patients
Combined	1	-	14	9	16	2	8	5
Calcium	1	-	9	4	2	12	6	2
Pentagastrin	2	-	5	2	5	4	5	5
Total number	4	0	28	15	23	18	19	12

Table 4: Duration of side-effects recorded during and after all the stimulation tests in the 10 patients with medullary thyroid carcinoma and in their 20 first-degree relatives.

Stimulation test	Time after the end of injection (min)*						Duration of side effect (min)	
	0.5	1	2	3	4	5	0.5-1	≥2
Combined	3	20	14	1	0	1	59%	41%
Calcium	1	4	10	0	2	0	29.4%	70.6%
Pentagastrin	0	3	8	0	4	0	20%	80%
Total number of side effects	4	27	32	1	6	1	43.7%	56.3%

preventive extensive cervical and mediastinal lymph-node dissection are associated with increased risk for complications and might not be necessary in some patients with MTC, particularly in tumors without desmoplastic stromal reaction²⁰. Intraoperative palpation of lymph nodes is not an accurate method for the detection of metastatic involvement (sensitivity 64% and specificity 71%⁸) and the resection of only the palpated lymph nodes that appear to be involved might not be successful in MTC. Therefore, the preoperative measurement of basal and post-stimulation CT levels might suggest the presence of micro-metastatic disease (in the cervical lymph nodes) and guide the decision for adjuvant operation. Another option would be the intraoperative assessment of CT levels but the long time (> 45 min) that is required for the measurement of CT concentration makes this approach less attractive^{21,22}. Another promising diagnostic approach is the combination of neck ultrasonography with the selective catheterization of the lower segment of the jugular veins to evaluate basal and post-Pg test CT levels³. This combination allows for the concurrent biochemical and imaging detection of minimal residual disease but is invasive and requires considerable operator experience, active participation of 3 medical doctors, patient consent and appropriate timing of blood sampling from both the peripheral and jugular veins. Then, targeted surgery by an experienced surgeon was performed, which could be curative.

To the best of our knowledge, this is the first study that compares 3 serum CT stimulation tests in post-thyroidectomy patients with minimal residual disease and in their first-grade relatives with C cell disease, even though it was not feasible to perform all 3 stimulation tests in all patients and in their relatives (only 14/30 subjects underwent all 3 stimulation tests). Accordingly, our study might have lacked the statistical power to detect differences in CT stimulation potency between the 3 tests and larger studies are needed to confirm or refute our findings.

In our study, all first-degree relatives of patients with MTC had normal basal serum CT levels. However, only the combined stimulation test identified abnormal serum CT levels (> 700 pg/ml) in 3 first-degree relatives in whom the Ca⁺² or Pg stimulation tests were non-suggestive of the presence of CCH. In these 3 patients, CCH was confirmed histologically after thyroidectomy. Peak serum CT levels in the remaining 17 first-degree relatives were within the normal range after all stimulation tests. In addition, in one patient with minimal residual disease and suspicious basal serum CT levels, only the combined and Pg stimulation tests yielded abnormal increases in CT levels whereas the Ca⁺² test was non-diagnostic.

In the present study, basal serum CT levels were abnormal in patients with clinically apparent metastatic disease. Therefore, these patients do not require stimulation tests. The variability in peak CT levels during the stimulation tests in these patients (which was observed in our study as well) has been attributed to the loss of expression of CCK-B/gastrin receptors in the neoplastic

C cell and to a large number of metastatic lymph nodes⁵. On the other hand, patients without residual disease had normal basal serum CT levels. In this group, CT levels were also normal after all 3 stimulation tests. Serum CT levels rose to suspicious levels (between 300-500 pg/ml) in one out of four patients without residual disease (25%) after the PG and the combined stimulation test, but not after the Ca⁺² stimulation test. Since MTC did not recur in this patient after a follow-up of 17 years, this finding could be regarded as a signal for low specificity of the Pg and combined stimulation tests.

Side effects were observed in the majority of tests (67/71, 93.8%) in 86.5% of all patients, but were of mild-to-moderate severity in the majority of cases, i.e. 76, 77 and 61% after the combined, Ca⁺² and Pg stimulation tests, respectively (Table 3). The majority of side effects (59%) during the combined stimulation test lasted for a very short time (≤ 1 min). In contrast, only 29.4 and 20% of side effects observed during the Ca⁺² or Pg stimulation tests, respectively, lasted ≤ 1 min (Table 4). All side effects resolved without intervention.

Other CT-secretagogues, such as omeprazole, are not as sensitive as Pg but can be used when the Pg test is contraindicated or not acceptable by the patient due to its associated side effects and in countries where Pg is not available^{11,12}. Indeed, Pg is unavailable in US, Japan and most European countries. In addition, a recent study in healthy volunteers showed that Ca⁺² exerts more potent CT stimulation and is better tolerated than Pg²³. Another recent study that compared the Ca⁺² and Pg stimulation tests in patients with MTC, carriers of RET gene mutations, patients with nodular goiter and healthy volunteers, confirmed the better tolerability of Ca⁺² even though the CT stimulation efficiency did not differ between the two tests²⁴. This study also reported a sensitivity of 90-100% and specificity, positive and negative predictive value of 100% for both the Pg and Ca⁺² stimulation tests²⁴.

As reported by many authors²⁵⁻²⁷, the overall sensitivity of CT screening was remarkably higher than that of FNAB, but marginal (suspicious) basal serum CT elevations alone eventually have limited value in diagnosing CCH or of a micro-C cell-disease.

In conclusion, patients with metastatic MTC have elevated basal serum CT levels and do not require stimulation tests. In contrast, basal serum CT levels can be normal in patients with minimal residual MTC and in subjects with CCH and these groups would require stimulation tests to establish the diagnosis. Compared with the isolated Ca⁺² or Pg tests, the combined short-lasting Ca⁺² plus Pg stimulation test appears to be more sensitive for the diagnosis of both minimal residual disease in patients who have undergone surgery for MTC (at an early stage) and of CCH in their first-degree relatives, when the surgical treatment can be curative. In addition, the combined test has comparable safety (short lasting side effects of mild-to-moderate severity) with the isolated tests. Therefore, the combined short-lasting Ca⁺² plus Pg stimulation test appears to be the most sensitive and safe method for

the early diagnosis of subclinical hypercalcitoninemia in patients with minimal MTC and their relatives with CCH.

Conflict of interest

Authors declare no conflict of interest.

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