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Stimulated Calcitonin Cut-Offs by Different Tests

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Medullary thyroid cancer · Calcitonin · Calcium · Pentagastrin · Cut-offs

Abstract
Medullary thyroid cancer can be highly aggressive, especially if the diagnosis is done in advanced stages. Early diagnosis is based on RET genetic testing, for familial forms, and on the routine measurement of calcitonin (Ct). Nevertheless, since false-positive results can be obtained with the basal measurement of Ct, a provocative test to evaluate stimulated Ct is often needed. Pentagastrin which has been widely used to stimulate basal Ct, especially in European countries, is now hardly available. Thus, the stimulation with calcium (Ca), used in the 1970s–1980s and then abandoned for around 30 years, has recently elicited more interest. In the past 3 years, studies in patients and normal controls have demonstrated that the stimulation with Ca (2.3–2.5 mg/kg of elemental Ca, corresponding to 25 mg/kg of Ca gluconate) is highly potent and accurate. Novel gender-related cut-offs have been proposed for the Ca test, though the analysis of additional large series is predicted to modify these preliminary data. Finally, Ca seems to be the test of choice to stimulate Ct for the diagnosis and follow-up of medullary thyroid cancer, also because it is widely available, has a low cost and it is associated with a low number and intensity of side effects. In the present review the different methods to stimulate Ct and the cut-offs for the identification of the hyperplastic/neoplastic transformation of the C cells will be reported and discussed.

Calcitonin, the Marker of Medullary Thyroid Cancer

Medullary thyroid cancer (MTC) can be a very aggressive tumor. Significant differences in survival have been reported according to the stage of the tumor at the time of diagnosis, the survival being similar to that of healthy subjects in the case of a local disease and progressively decreasing in case of local or distant metastases [1]. Thus, an early diagnosis is mandatory for this malignancy. The routine measurement of calcitonin (Ct) was initially indicated in 1994 as the best method, together with the RET genetic testing for familial forms, to precociously identify sporadic forms of MTC. In particular, among more than

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1,300 patients with nodular thyroid disease, the routine measurement of Ct allowed to identify 8 MTCs, only 2 of whom were diagnosed also by a suggestive cytology [2]. After that study, many others, for a total of more than 70,000 cases, reported the results obtained with the routine measurement of Ct, with a mean prevalence of MTC of 0.5%. Interestingly, around half of the MTCs found were micro-MTCs, confirming the importance of the routine measurement of Ct in the early diagnosis of C-cell diseases [3]. The impact on survival was clearly demonstrated in a study including 44 patients (group 1) diagnosed with MTC by the routine Ct measurement and 45 patients diagnosed by other methods (group 2). A statistically significant difference in the rate of complete remission was found between the two groups of patients (59% in group 1 and in 2.7% in group 2, $p < 0.0001$), highlighting that the routine Ct measurement should always be considered in the diagnostic evaluation of thyroid nodules [4, 5]. Nevertheless, this practice remains controversial and is definitely accepted only for the screening of familial MTC. In particular, the European Consensus recommended it, whereas the American Thyroid Association guidelines do not recommend either for or against it [6, 7]. Moreover, the recent American-European-Italian joined guidelines consider that the testing of Ct may be a useful test in the initial evaluation of thyroid nodules, but do not recommend its routine use [8].

Routine Measurement of Calcitonin: Why Not?

The major arguments against the routine measurement of Ct are reported below:

(1) Costs/benefits. The prevalence of MTC is low, around 0.5% of the population with thyroid nodules. For this reason it has been argued that the routine testing of serum Ct in all patients with unselected thyroid nodules could not be cost-effective. Nevertheless, a very detailed study has recently demonstrated that this test has a cost/benefit ratio similar to those of TSH neonatal screening, and colonoscopy and mammogram screening [1].

(2) Uncertain evolution of micro-MTCs. Since around half of the tumors identified by routine Ct measurement are micro-MTCs, it has been argued that these tumors could have a highly reduced malignant potential and will never become larger and aggressive tumors. Indeed the prevalence of MTCs in unselected autopsies is roughly comparable to the Ct screening studies in the thyroid nodule population [3].

(3) Risk of false-positive results. These are mostly due to assay interferences, such as the presence of heterophilic antibodies that can cause falsely elevated and rarely falsely reduced Ct levels. Nevertheless, these interferences are usually abolished by the use of immunometric chemiluminescent assays two-site, two-step (ICMAs), highly specific for monomeric Ct.

(4) Presence of secondary C-cell hyperplasia, which can be related to: (a) thyroid diseases: autoimmunity (in 20% of cases), benign or malignant nodules; (b) other diseases: severe renal insufficiency, hyperparathyroidism, hypergastrinemia.

(5) Increased basal Ct secretion during treatment with drugs, such as omeprazole, β-blockers, glucocorticoids secretagogues and cigarette smoking [9, 10].

(6) Production of Ct by neuroendocrine tumors. In this context, it should be underlined that neuroendocrine tumors usually produce less Ct per gram of tissue than MTC and typically do not increase their Ct secretion in response to the stimulation testing.

(7) Difficulties in the set-up of the cut-offs for both basal and stimulated Ct needed to identify the C-cell diseases.

(8) Unavailability in the USA, and now also in Europe, of pentagastrin (Pg) which has been considered for several years as the best test to stimulate Ct.

The Cut-Offs for Pg Test

The Pg test has largely been used for many years to stimulate Ct. The procedure consists of the injection of 0.5 μg/kg over 5 s and in the basal, +1–2, +5 and +10–15 min sampling. Several patients have been tested and many studies have been published with the aim to establish the most precise cut-offs to differentially diagnose between normal, C-cell hyperplasia (CCH) and MTC [11–15]. The results of these papers have been summarized in table 1. The indication for surgery is almost widely accepted for Ct-stimulated levels >100 pg/ml, whereas the best treatment for cases with stimulated Ct >50 pg/ml is still controversial. Interestingly, in a recent study we demonstrated that, in patients with multinodular goiter, Pg-stimulated Ct levels >50 pg/ml were always associated with a diffuse/nodular CCH. Moreover, in all cases the C cells displayed a neoplastic phenotype, concerning morphology, distribution and localization, indistinguishable from that found in familial MTC and thus considered as a preneoplastic lesion. According to those findings, in patients with Pg-stimulated Ct levels >50 pg/ml a 'prophy-

Table 1. Cut-offs for basal and Pg-stimulated Ct

	Interpretation	Indication
Basal Ct		
≤10–20 pg/ml	normal	–
10–50 pg/ml	gray zone	stimulation test
50–100 pg/ml	risk of MTC	stimulation test
Pg-stimulated Ct		
<30–50 pg/ml	normal	–
50–100 pg/ml	CCH	surgery
>100 pg/ml	risk of MTC	surgery

lactic' surgical treatment should be considered [16]. Nevertheless, it should be highlighted that the peak value of stimulated Ct should be interpreted not only as an absolute value, but also taking into account the basal level of Ct and, more importantly, the degree of Ct increase after stimulus. Indeed, MTC has been found to be associated with a minimum percent increment of 160% [2].

Why It Is So Difficult to Establish a Definite Cut-Off

There are some reasons to explain the difficulties in the definition of the cut-offs.

(1) Different assays in different centers. Although international guidelines recommend each center to establish its own cut-off values for basal and stimulated Ct [6, 7], the existence of different ranges largely contributes to the difficulties in the identification of definite basal and stimulated Ct levels to differentiate between normal and C-cell diseases.

(2) Lack of different children- and gender-related ranges. Although Ct values in children are believed to be higher than in adults, American Thyroid Association (ATA) guidelines report that due to the limited data available, caution should be used in interpreting Ct values in children <3 years of age [10]. In a large cohort of healthy subjects we found that the normal range of serum Ct levels was wider in children (<0.2–11.7 pg/ml for female and <0.2–17 pg/ml for males) than in adults, without significant gender differences in the children population [18]. In adults, Ct values should be interpreted in the setting of sex-specific reference ranges, as suggested by ATA guidelines [10], though most centers to date have a unisex threshold for Ct levels. Indeed, since men physiologically have twice as many C cells as women [18], both basal and stimulated Ct secretion are gender-related [9]. In this context, it has been demonstrated that gender-specific Ct thresholds predict occult MTC more accurately among patients with increased basal Ct levels than unisex thresholds. In particular, the most discriminatory threshold between CCH and MTC was found to be for basal Ct of 15 pg/ml for women and 80 pg/ml for men, and for Pg-stimulated Ct of 80 pg/ml for women and 500 pg/ml for men [12].

The Calcium Test

The unavailability of Pg in the United States and recently also in most European countries recently raised the need to standardize other methods to stimulate Ct. The calcium (Ca) test was seldom used in the 1970s and 1980s, but has been abandoned in the last 30 years (table 2). There are some basic reasons for the removal of the Ca test from clinical practice: (1) different doses in different protocols: from 2 up to 15 mg/kg of elemental Ca (Ca^{2+}); (2) lack of a precise specification of the dose of Ca^{2+} (to be obtained from the starting solution, i.e. from Ca gluconate of Ca chloride); (3) different times of injection: from 1 min to 4 h; (4) use of protocols including the injection of Pg and Ca on consecutive days [19] or Ca together with Pg [20], and (5) no data available on the cut-off levels to be used for the preoperative identification of a CCH or a MTC.

Moreover, the Ca test was initially performed as a long Ca infusion, with 3.75–5 mg/kg/h for a total duration of 3–4 h. This procedure had the disadvantage of the large amount of Ca infused, usually around 1.5 g, which produced a significant and prolonged elevation of plasma Ca, rendering it not acceptable for the screening of a large number of family members [21]. In addition, discordant data were available about the potency of the test, though the majority of studies indicated that Pg had a better stimulator than Ca. In particular, Hennessy et al. [20] and Verdy et al. [22] in 1974 suggested that Pg had a more rapid and intense secretory response than a 2- or 4-hour infusion of Ca^{2+}. In 1980, Emmertsen et al. [23] stated that Pg was a better stimulative procedure than the rapid infusion of 2 mg/kg of Ca^{2+}. In 1978, Wells et al. [24] demonstrated that the combination of Ca^{2+} and Pg was more effective than Ca^{2+} and Pg alone. In 1987, Gharib et al. [25] found Ca^{2+} injection (2 mg/kg over 5 min) to be more potent than Pg in healthy volunteers and weaker in a small group of thyroidectomized MTC patients. The data available to date on the Ca test protocols reported in the

Table 2. Ca test protocols in the literature

Reference (first author)	Year of publication	Number of subjects/patients	Infused dose of the starting solution[1]	Infused dose of elemental Ca[1]	Time of i.v. infusion	Saline
Hennessy [20][2]	1974	38 patients	Ca gluconate: 161 mg/kg	15 mg/kg	4 h	500 ml
Parthemore [31]	1974	4 patients	Ca chloride: 550 mg	150 mg	5–10 min	50 ml
Sizemore [32]	1975	4 patients	Ca gluceptate	15 mg/kg	4 h	500 ml
Parthemore [21]	1978	11 normal controls 10 normal controls	Ca chloride: 11 mg/kg Ca gluconate: 22 mg/kg	3 mg/kg 2 mg/kg	10 min 1 min	–
Verdy [22]	1978	39 family members	Ca gluconate: 81/mg/kg	7.5 mg/kg	2 h	250 ml
Graze [33]	1978	107 familial members	Ca gluconate: 161 mg/kg	15 mg/kg	4 h	–
Wells [24]	1978	21 normal subjects 26 MTC patients	Ca gluconate: 22 mg/kg Ca chloride: 11 mg/kg	2 mg/kg 3 mg/kg	1 min	–
Emmertsen [23]	1980	6 patients with persistent MTC	Ca laevulatis	2 mg/kg	1 min	–
McLean [34]	1984	31 healthy subjects 13 patients with MTC	Ca chloride: 11 mg/kg	3 mg/kg	5 ml/min	50 ml
Gharib [25]	1987	92 normal controls 12 patients with MTC	Ca gluconate: 22 mg/kg	2 mg/kg	1 ml/min	50 ml
Doyle [26][3]	2009	50 healthy subjects	Ca gluconate: 27 mg/kg	2.5 mg/kg	10 ml/min	–
Kudo [35]	2011	20 patients with thyroid diseases other than MTC	Ca gluconate: 20.2 mg/kg	1.9 mg/kg	1 min	–
Colombo [28]	2012	56 patients with persistent/cured MTC 60 MNG patients; 16 healthy subjects	Ca gluconate: 25 mg/kg	2.3 mg/kg	10 ml/min	–
Giovanella [36]	2012	96 healthy subjects	Ca gluconate: 2.5 mg/kg	0.2 mg/kg	10 ml/min	–

[1] Both the dose of Ca gluconate or Ca chloride and the corresponding dose of elemental Ca have been reported (according to the Ca equivalents calculator on the website http://www-users.med.cornell.edu/~spon/picu/calc/cacalc.htm).

[2] The infusion of Ca was preceded or followed by the injection of Pg (0.5 μg/kg).

[3] Note: The authors requested to change the following text reported in the Materials and Methods section of the original paper [J Clin Endocrinol Metab 2009;94:2970–2974]: '*... calcium gluconate (Calcium Braun 10%; Braun Melsungen AG, Melsungen, Germany) as an intravenous injection of 2.5 mg/kg at 10 ml/min,*' to: '*... calcium, 2.5 mg/kg, was given as a calcium gluconate solution (Calcium Braun 10%; Braun Melsungen AG, Melsungen, Germany) containing approximately 9 mg/ml calcium, injected intravenously at 10 ml/min*' [erratum in J Clin Endocrinol Metab 2009;94:4629].

literature are shown in table 2. The dose of Ca gluconate or Ca chloride has also been reported together with the corresponding dose of Ca^{2+}, according to the Ca equivalents calculator on the website http://www-users.med.cornell.edu/~spon/picu/calc/cacalc.htm. As stated above, the test was abandoned for many years up until it was re-evaluated in 2009 by Doyle et al. [26], who reported data in 50 healthy subjects. They decided to use a dose of 2.5 mg/kg of elemental Ca in accord with the dose of 2–3 mg/kg which was the most frequently used in the 1970s and 1980s (table 2). The authors concluded that the Ca test is more potent and better tolerated than Pg. In particular, they found that the peak of Ct after Pg injection is around 10 pg/ml for males and 4 pg/ml for females, consistent with previous results reporting Pg-stimulated values of around 15 and 5 pg/ml, respectively [27]. On the other hand, the levels of Ct after Ca^{2+} stimulation resulted in being higher, around 40 pg/ml for men and 20 pg/ml for women. To extend the results obtained in healthy subjects and to get more insight into the standardization of the Ca^{2+} test, we recently published the results obtained in controls, in patients with multinodular goiter and in patients with both familial and sporadic MTC, either in persistence or in remission [28]. As far as normal controls are concerned, peak serum Ct levels after Ca infusion did not significantly differ between men and women (around 20 and 15 pg/ml, respectively). In 4 of 16 controls, both tests were carried out and similar responses between Pg and Ca^{2+} were obtained in 3 patients whereas in 1 female only Ca^{2+} was able to stimulate a response of Ct (fig. 1),

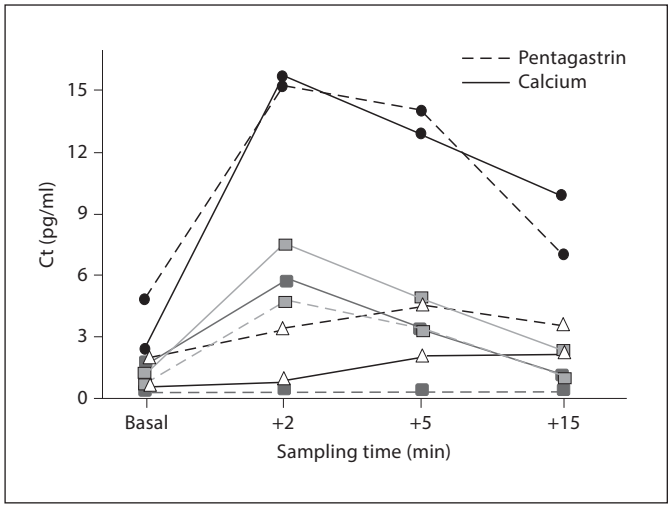

Fig. 1. Basal and Pg/Ca-stimulated levels of Ct in 4 normal subjects who underwent both tests. Similar responses were obtained in 3 cases, whereas in 1 case only Ca was able to induce a rise in Ct levels.

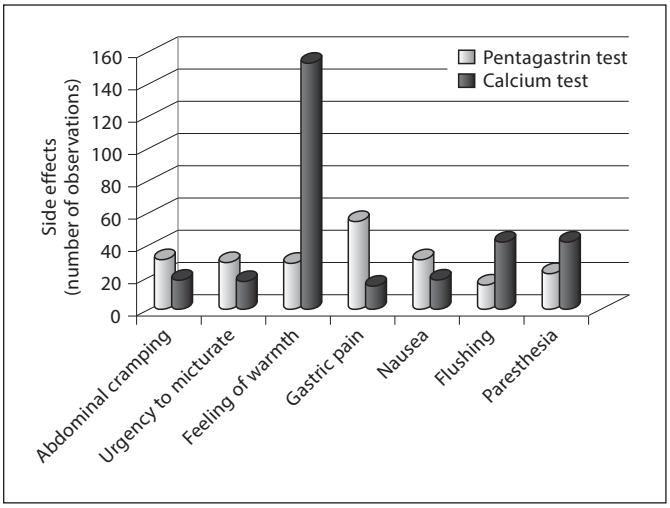

Fig. 2. Side effects reported by patients during either Pg or Ca tests.

consistent with the finding of Doyle et al. [26] who reported a higher potency of Ca^{2+} with respect to Pg. In patients with MTC and nodular goiter, we found a nice correlation between the two tests, without statistically significant differences in the levels of Pg or Ca^{2+} serum Ct in patients submitted to both tests. Nevertheless, serum Ct levels have been found to be higher after Ca^{2+} than after Pg in several patients, indicating that studies in larger samples could reveal a higher potency of the Ca test. ROC plot analyses were used to find the basal and Ca^{2+}-stimulated Ct thresholds able to differentiate between normal, CCH, and MTC, for men and women. As reported in table 3, all patients with basal Ct levels >10 pg/ml were submitted to the Ca^{2+} test and the levels of basal and stimulated Ct were compared with the histological findings. The results obtained showed that the Ca^{2+}-stimulated Ct thresholds able to distinguish normal controls and CCH cases from patients with MTC were 184 pg/ml for females and 1,620 pg/ml for males [28]. Concerning the degree of Ct elevation after stimulus, MTC was associated with Δ increases ranging from 5.6 to 64.8 for females to 5.9 – 92.3 for males (table 3).

Calcium Test: Procedure and Side Effects

To date, the paper by our group is the only one to report the cut-offs for Ca-stimulated Ct [28]. The Ca test was performed according to the following procedure: Ca gluconate was administered intravenously at a dose of 25 mg/kg at 10 ml/min (this corresponds to 2.3 mg/kg or 0.12 mEq/kg of elemental Ca). In clinical practice it is recommended to use 10% Ca gluconate: each 10-ml vial contains 950 mg monohydrate of Ca gluconate (corresponding to 88.3 mg or 4.41 mEq of elemental Ca). Thus, for a 50-kg subject the dose of Ca gluconate needed is: 25 × 50 = 1,250 mg. The following calculation will be: 950:10 = 1,250:x (x = 13.15 ml of 10% Ca gluconate solution). Serial measurement demonstrated that at 2 min after Ca infusion, ionized Ca levels increase by 30–35% and then progressively decrease, whereas PTH levels decrease by 50–60% and continue to decrease. 30 min after high Ca infusion, Ca levels were 5–10% higher than baseline and PTH levels were 65–70% lower than baseline.

Overall, the number, intensity and duration of side effects were significantly lower during the Ca test and all patients declared that they preferred it. Nausea and abdominal cramping were the most frequent discomforts during the Pg test and were poorly tolerated by patients. On the contrary, the feeling of warmth, which was the most frequent and often the only side effect with Ca test, was not considered unpleasant (fig. 2).

Ca is known to increase cardiac contractility and it could lead to hypertensive peaks. On the other hand, hypertension can cause bradycardia. Thus, anamnestic data related to cardiopathy or bradycardia or severe hypertension should be obtained by patients. In selected cases, the

Table 3. Clinical and histological data of patients with basal Ct levels >10 pg/ml. Adapted from Colombo et al. [28]

Age/ gender	Basal Ct pg/ml	Ca Ct peak pg/ml	Δ increases	Histology	Tumor diameter, mm	TNM	CCH
40/F	15/19	184	9.7	UNG	–	–^{thy}	yes
60/F	59/29	331	5.6	MTC	7	pT1Nx^{thy}	no
37/F	40	353	8.8	MTC	10	pT1N1a^{thy}	yes
44/F	16	522	32.6	UNG	–	–	yes
56/F	44.5	1,483	33.3	MTC	6	pT1N0^{thy}	no
76/F	68/57	1,524	22.4	MTC	10	pT1Nx	yes
73/F	121	1,801	14.9	MTC	21	pT1Nx	yes
49/F	126/164	2,349	14.3	MTC	11	pT1N0	yes
53/F	592	38,362	64.8	MTC	19	pT1N0	yes
58/M	10	190	19	MNG	–	–	yes
64/M	14/12	196	14	MNG	–	–	yes
43/M	16/11	205	12.8	PTC	2	pT1Nx	yes
54/M	16	263	16.4	MNG	–	–	yes
70/M	17/21	368	17.5	MNG	–	–	yes
50/M	94/87	562	5.9	MTC	7	T1aNx	not done
71/M	23/23	806	35	PTC	10	pT1Nx	yes
70/M	95/68	1,620	17	UNG	–	–^{thy}	yes
65/M	218/198	2,900	13.3	MTC	17	pT1Nx	yes
56/M	73	5,846	80	MTC	13	pT1Nx	yes
60/M	154/170	15,700	92.3	MTC	14	pT1Nx	not done

MNG = Multinodular goiter; UNG = uninodular goiter; PTC = papillary thyroid cancer; thy = associated thyroiditis.

test can be done under cardiac monitoring. Nevertheless, in our hands, neither electrocardiographic changes nor heart rate variations have ever been observed. The test has also been performed without any side effects in hyperparathyroid patients [28].

Other Tests

Scanty data are available on other tests for Ct stimulation. In a small series of 6 patients and 8 controls, the stimulus with whisky resulted in being less efficient than Pg and Ca [23]. Omeprazole has also been used to stimulate Ct at a dosage of 20 mg twice daily for 3–4 days, with blood sampling every morning [29, 30]. Although a steady and significant increase in Ct levels was achieved, omeprazole appeared to be less potent and sensitive than Pg. Thus, its use should be limited to cases where Pg or Ca^{2+} cannot be performed.

Discussion

MTC is a potentially highly aggressive tumor and the diagnostic/therapeutic tools available to date are limited and not sufficiently sensitive or effective. Thus, an early diagnosis is mandatory, which can be achieved by RET genetic testing and routine Ct measurement. In particular, Ct (basal and stimulated) allows early diagnosis either pre- or post-surgery. The majority of studies have been performed using the stimulus with Pg and cut-offs for Pg-stimulated Ct are available. Unfortunately, Pg has virtually become unavailable. For this reason, in the last 2 years the Ca test, which was abandoned in the last 30 years, has been demonstrated to represent a good choice to stimulate Ct. Some data are available on the stimulation response in normal subjects and in patients with Ca gluconate doses of 25 mg/kg, corresponding to 2.3 mg/kg of elemental Ca [26, 28]. In these studies, Ca has been found to be equivalent or superior to Pg in the stimulation of Ct for the diagnosis and follow-up of MTC. Moreover, cut-off values to differentially diagnose among

CCH and MTC in either males or females have been recently reported by our group [28]. Nevertheless, to better refine the cut-off values, more series tested with the same Ca protocol are definitely needed. In conclusion, Ca is widely available, has a low cost and it has been demonstrated to have at least the same potency of Pg and should thus be used for the diagnosis and follow-up of MTC. The personal final auspice of the author is that the availability of Ca and the easiness of execution of the test could render the routine measurement of Ct more widely used.

Disclosure Statement

The author has no conflicts of interest to disclose.

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