

Refining Calcium Test for the Diagnosis of Medullary Thyroid Cancer: Cutoffs, Procedures, and Safety

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Context: Calcitonin (CT) measurement is crucial to the early diagnosis and the follow-up of medullary thyroid cancer (MTC). If the evaluation of stimulated CT levels is required, a provocative test can be performed, being the high-dose Ca test recently reintroduced in clinical practice.

Objective: Our objective was to identify gender-specific thresholds for MTC diagnosis in a large series of patients who underwent the Ca test.

Patients and Methods: A total of 91 patients (49 females and 42 males) underwent the Ca test (calcium gluconate, 25 mg/kg) before thyroidectomy and both basal CT (bCT) and stimulated CT (sCT) were compared with histological results by receiver operating characteristic plot analyses. To evaluate possible side effects of Ca administration, cardiac function has been extensively studied.

Results: bCT levels were found to harbor the same accuracy as sCT in the preoperative diagnosis of MTC. The best Ca thresholds for the identification of MTC were >26 and >68 for bCT and >79 and >544 pg/mL for sCT in females and males, respectively. The high tolerability and safety of the Ca test was demonstrated and advice offered to be followed before and during the test.

Conclusions: Gender-specific bCT and sCT cutoffs for the identification of C-cell hyperplasia and/or MTC have been defined. The bCT and sCT were found to have a similar accuracy, indicating that serum CT assays with improved functional sensitivity may likely decrease the relevance of the stimulation test in several conditions. Finally, systematic cardiac monitoring confirms the safety of the Ca test. (*J Clin Endocrinol Metab* 99: 1656–1664, 2014)

Medullary thyroid carcinoma (MTC), originating from the parafollicular C cells of the thyroid, can display an aggressive behavior especially if diagnosed in an

advanced stage (1). The measurement of serum calcitonin (CT) constitutes the best method, together with the RET genetic testing for familial forms, to precociously identify

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Abbreviations: AUC, area under the ROC curve; bCT, basal CT; CCH, C-cell hyperplasia; CT, calcitonin; ECG, electrocardiographic; FA, follicular adenoma; FTC, follicular thyroid carcinoma; IQR, interquartile range; MNG, multinodular goiter; MTC, medullary thyroid carcinoma; Pg, pentagastrin; PTC, papillary thyroid carcinoma; ROC, receiver operating characteristic; sCT, stimulated CT.

sporadic forms of MTC, with a major impact on survival (2). CT should be measured by highly specific immunometric chemiluminescent 2-site, 2-step assays to avoid heterophilic antibody interference causing a false hypercalcitoninemia (3). The finding of CT levels higher than the normal range during the initial evaluation of thyroid nodules may require a confirmatory stimulation test. In particular, the test can be useful in the following conditions: 1) to identify the possible coexistence of nonthyroidal neuroendocrine tumors of the foregut, pancreas, prostate, and lung that can be distinguished from a C-cell disease by the absence of response to the stimulation test; 2) to differentiate MTC from C-cell hyperplasia (CCH), the preoperative recognition of which should avoid unnecessary thyroidectomies because this reactive CCH is very likely a benign condition frequently associated with thyroid diseases other than MTC (autoimmune thyroiditis or benign nodules) or to other diseases (severe renal insufficiency, hyperparathyroidism, or hypergastrinemia); and 3) to achieve a preclinical early diagnosis of neoplastic CCH or micro-MTC and perform a prophylactic thyroidectomy in *RET* mutation carriers. The stimulation test has been performed by pentagastrin (Pg) iv administration for several years, but its unavailability in the United States and recently also in most European countries, raised in the last 2 to 3 years the need to standardize other methods to stimulate CT. The Ca test was seldom used in the 1970s and 1980s, but it was abandoned during the last 30 years, for several reasons including the lack of standardization of the test in terms of doses and duration of the Ca administration (4). Moreover, no data were available for the Ca test on the cutoff levels to be used for the preoperative identification of CCH or MTC. A different mode of action has been reported for Pg and Ca. In particular, Pg induces the release of CT from C cells by binding cholecystokinin B/gastrin receptors, which are expressed in both normal C cells and in MTC (5). On the other hand, a stimulated CT (sCT) test obtained through calcium infusion is based on the fact that C cells express the same extracellular calcium-sensing receptor that is found in parathyroid and kidney (6). The calcium-sensing receptor represents the primary molecular entity through which C cells detect changes in extracellular calcium concentrations and is able, in response to acute increases in ionized calcium, to stimulate CT release (7, 8). The Ca test has been recently reevaluated in healthy subjects (9), in patients with multinodular goiter and with either familial or sporadic MTC (10), and in thyroid conditions other than MTC (11). The Ca dose selected was of 2.3 to 2.5 mg/kg elemental Ca, according to the dose of 2 to 3 mg/kg, which was the most frequently used in the 1970s and 1980s (4). The Ca test was found to be more potent and better tolerated than the Pg test (9–

11), and the maximum Ca- and Pg-stimulated CT levels were significantly correlated (10). Our previous study used receiver operating characteristic (ROC) plot analyses to define for the first time the basal CT (bCT) and sCT thresholds able to differentiate between normal, CCH, and MTC for men and women. We showed that the bCT levels with the best accuracy to identify MTC were >18.7 pg/mL in females and >68 pg/mL in males. On the other hand, the best value of sCT to distinguish non-MTCs (normal controls and CCH) from MTCs were 184 pg/mL for females and 1620 pg/mL for males (10). Nevertheless, the number of patients suitable for the ROC analyses (ie, with available Ca test and histological data) was limited, due to the rarity of the disease, to 25 females and 15 males. Thus, the present study aims to refine in a larger series, which includes data from the previously published patients (10), the cutoff levels for CT, both bCT and sCT, for a better differential diagnosis between normal, CCH, and MTC.

Patients and Methods

A total of 91 patients (49 females and 42 males) were enrolled for the present study. In particular, 50 patients (24 females and 26 males, age range 21–81 years) had a nodular goiter and bCT levels ≥ 10 pg/mL, whereas 32 (20 females and 12 males, age range 20–77 years) had bCT <10 pg/mL and an indication for total thyroidectomy due to major tracheal compression or to cytology results (indeterminate or suspicious for papillary thyroid cancer). Moreover, a group of 9 patients (5 females and 4 males, age range 8–45 years) known to be carriers of a *RET* mutation (*RET* S891A in 4 females and in 3 males, *RET* C634G in 1 female and in 1 male) underwent the stimulation test regardless of the bCT levels. All patients gave their informed consent to be enrolled in this study, which has been approved by the ethical committees of the institutions involved. All patients underwent total thyroidectomy associated or not with lymphadenectomy in accordance with the preoperative diagnosis.

Procedure

In consideration of the proarrhythmic risks linked to the iv administration of calcium gluconate loads, a preventive evaluation of some clinical, instrumental, and biochemical aspects of each patient is warranted. Anamnestic data must be focused on cardiac diseases and on the possible assumption of cardiac glycosides because Ca could potentiate their effects. The electrocardiographic (ECG) evaluation must test the heart frequency and the QT and PR intervals because a frequency of <40 or >110 beats/min and/or the presence of second or third degree atrioventricular block contraindicates the execution of the test. Before the test, it is recommended to test that ionized calcium, potassium, phosphate, and magnesium levels lie in the normal range. Indeed, hyperphosphatemia could cause calcium and phosphate precipitation, calcium and magnesium act as antagonists of each other in blood vessel tone, and calcium determines variation in potassium plasma concentrations with potential consequences on the QT interval. In case of ion alteration, an appropriate correction is recommended before performing the test.

If there are no contraindications to the test, a valid peripheral venous access must be placed, the blood pressure must be recorded, and electrodes for heart rate and ECG detection must be applied and maintained during the test. The recommended dose of Ca gluconate is 25 mg (2.3 mg or 0.12 mEq of elemental calcium)/kg, but the adjusted body weight must be calculated (www.manuelsweb.com/IBW.htm for ideal body weight and adjusted body weight calculator) for each patient to avoid an overdose in the obese. After a basal blood sampling for CT, Ca gluconate must be administered iv at 5 mL/min, with a minimum time of administration of 3 minutes. By the end of the infusion, additional blood samples must be taken at minute +2, +5, and +10. In some patients, a Holter ECG was applied for about 12 hours to highlight possible arrhythmias. Moreover, to investigate the heart muscle performance during Ca infusion, a standard and advanced tissue Doppler echocardiography was done. CT was measured using a 2-site automated chemiluminescent immunometric assay (Immulite 2000; Siemens Diagnostics). The assay has an analytical sensitivity of 2 pg/mL.

Immunohistochemical studies

In 80 of 91 cases, a minimum of 6 paraffin blocks (3 from the left and 3 from the right lobes corresponding to upper, intermediate, and lower zones) were selected, excluding extensive fibrotic or hemorrhagic areas. Additional and unlimited sampling was done in the case of multinodular goiters or when even minimal lesions were detected at macroscopic examination. From each paraffin block, additional 5- μ m sections were obtained and tested for CT reactivity, as previously described (10). In particular, CCH was defined as more normal-appearing C cells (at least more than 50 C cells per low-power field) (12, 13). According to the growth pattern of C cells, CCH was morphologically defined as linear, diffuse, or nodular (14, 15). In some cases, the C-cell count was obtained.

Statistical analyses

The bCT and sCT levels are reported as mean \pm SD. The sCT levels have been also reported as median and interquartile ranges (IQRs), and data were log-transformed for statistical analysis. The cutoff levels corresponding to the highest accuracy to differentiate between normal, CCH, and MTC were assessed by the ROC curves. It is worth noting that the data obtained in the present series were pooled with those already reported by our group (10) to increase the potency of the analysis. Statistical significance was defined as $P < .05$. All statistical analyses were performed using SPSS version 8.0 for Windows and MedCalc Software version 11.6.1 for Windows.

Results

Patients with basal CT levels <10 pg/mL

Thirty-two patients with bCT levels <10 pg/mL and committed to a total thyroidectomy for reasons other than a suspicion of MTC underwent the Ca test. Two females did not show any CT response to the test, whereas in the remaining patients, the mean peak response was 27.7 ± 28.6 (range 3.3–115) pg/mL in females and 69.4 ± 78.4 (range 2.9–229) pg/mL in males. The histological exam-

ination showed a benign multinodular goiter (MNG) in 10 females and 5 males, a follicular adenoma (FA) in 2 males, a papillary thyroid carcinoma (PTC) not preoperatively diagnosed in 10 females and in 4 males and a follicular carcinoma (FTC) in 1 male. In 3 females and 5 males, an associated CCH was found (Tables 1 and 2).

Patients with basal CT ≥ 10 pg/mL

After the Ca stimulation test, the 50 patients with nodular goiter and basal CT levels ≥ 10 pg/mL underwent total thyroidectomy, associated or not with pretracheal/paratracheal lymphadenectomy. The mean bCT and sCT levels were 35.9 ± 32.8 (range 10.3–128) and 622.5 ± 876.4 (range 12.7–3573) pg/mL in females and 176 ± 382.5 (range 10.2–1860) and 2350.7 ± 4729.7 (range 130–20 000) pg/mL in males, respectively. Because the distribution of peak CT was not Gaussian and included extremely high values, the logarithmic (log) transformation was applied. Median sCT levels were significantly higher in males (median 552.9, IQR 288–1620) than in females (median 288.9, IQR 105–638) ($P = .005$ after log-transformation). Similarly, the Δ -increases (ie, the difference between the sCT and the bCT level) were higher in males (median 502.8, IQR 273–1552) than in females (median 264.6, IQR 75–599) ($P = .005$ after log-transformation). The histological examination showed an MTC in 10 females and in 11 males, associated with CCH in 4 cases. Interestingly, in 4 cases (3 females and 1 male), the MTC was associated with a PTC. In the remaining cases, histology showed an MNG in 9 females and 6 males (associated with CCH in 14 cases), an FA in 1 female, a PTC in 4 females and in 8 males (associated with CCH in 9 cases), and an FTC in 1 male (Tables 1 and 2).

RET gene carriers

Nine patients harboring a *RET* mutation underwent the stimulation test before undergoing total thyroidectomy, associated with pretracheal/paratracheal lymphadenectomy. Three patients had bCT levels <10 pg/mL with a peak response ranging from 95 to 224 pg/mL, whereas the other 6 cases had bCT levels >10 pg/mL with peak response ranging from 135 to 26 000 pg/mL. The histological examination showed MTC in 8 of 9 cases, associated with CCH in most of them (Tables 1 and 2). It is worth noting that 2 gene carriers with bCT <10 pg/mL (V.V.: bCT 5.7, sCT 127 pg/mL in Table 1; R.U.: bCT 9.5 and sCT 224 pg/mL in Table 2) were found to harbor an MTC at histology. On the other hand, B.M. (Table 2) had a bCT of 7.7 and a peak response of 95.2 pg/mL, without MTC or CCH at histology.

Table 1. Clinical and Histological Data of Female Patients (n = 49)

ID/Age, y	CT Pre-Tx, pg/mL		Histology (mm) ^{cytology}	TNM (Metastatic lfn/Total lfn)	CCH
	Basal	Peak			
bCT <10 pg/mL					
C.C./40	<2	<2	PTC	pT1amNX	No
P.M./77	<2	<2	PTC	pT1NX	No
S.I./36	<2	3.3	MNG		No
S.G./23	<2	4.9	MNG		NA
G.E./32	<2	5.5	PTC	pT2NX	No
M.F./61	<2	5.6	PTC	pT1NX	L
S.G./60	<2	6.2	PTC	pT1NX	No
G.G./38	<2	9.5	MNG		No
Z.A./37	<2	15.3	MNG ^{thy}		No
M.A./63	2.9	18.5	MNG		NA
S.R./67	<2	18.9	MNG ^{thy}		D
B.A./35	2.2	22.4	MNG		NA
R.G./65	2.1	23.5	MNG		No
C.B.R./57	2.1	26.5	PTC	pT1mNX	No
F.L./50	3.2	27.2	PT ^{thy}	pT1NX	No
S.A./47	1.7	32	MNG ^{thy}		No
G.M./35	3.2	34.5	PTC	pT3NX	NA
L.J./36	2	55.2	MNG		No
M.L./74	7.3	74.8	PTC	pT1NX	D
M.R./33	6	115	PTC	pT1NX	No
V.V./32, #1	5.7	127	fMTC (2) nd	pT1aN0 (0/12)	NA
bCT >10 pg/mL					
C.F./22	11.9	12.7	MNG		No
F.C./45	15.9	18.5	PTC	pT3mN1a	No
B.C./33	12.2	64.5	PTC ^{thy}	pT1mNX	L
D.L./59	16	76	PTC + lung carcinoid	pT1NX	L
V.M./71	25.8	79	MNG		L
B.M./54	13.7	88.1	sMTC (2.3) nd	pT1aN0 (0/6)	No
P.E./66	50	126	sMTC (6) nd	pT1aN1a (1/3)	No
M.E./40	13.4	130	MNG		L
B.G./59	11	156	PTC	pT1NX	D
B.M.L./45	15	247	MNG		L, D, N (76.7)
B.A./58	15.6	266	MNG		L, D, N (39.8)
R.D./71	62.8	288	sMTC (7) ^{ind}	pT1aN0 (0/8)	No
T.M.E./58	10.3	290	FA		NA
R.S./21, #1	15	360	fMTC (4) nd	pT1mNX	L, D, N (72.3)
C.C./26, #1	15.5	312	fMTC (2.5) nd	pT1aN0 (0/5)	No
C.A./60	29	331	sMTC (7) nd	pT1N0 (0/6)	No
Z.A./57	32	358	PTC + sMTC (11) ^{susp}	pT1NX/pT1bN0 (0/11)	No
P.E./61	25.7	431	MNG		N
L.A./51	15.6	522	MNG		L, D, N (57.3)
I.C.H./45	19.9	599	MNG		N
R.L./50	58.8	679	sMTC (6) nd + PTC	pT1N0 (0/6)/pT1N0	No
V.T./37, #2	39.9	807	fMTC (6) nd	T1mN0 (0/7)	N
T.J./38	39	1070	MNG ^{thy}		L, D, N (97.3)
M.E./66	44.3	1130	sMTC (5) nd	pT1NX	L (20.6)
R.A./73	121	1906	sMTC (21) nd	pT2N0 (0/8)	L, D, N (93)
P.L./70	128	2500	sMTC (18) ^{neg} + PTC	pT1NX, T1mNX	NA
D.T.A./56	75	3573	sMTC (8) ^{neg}	pT1NX	L, D, N (59)
B.C./45, #1	520	26 086	fMTC (25) nd	pT3mN1b (4/12)	L, D, N (98.4)

Abbreviations: #1, gene carrier *RET* S891A; #2, gene carrier *RET* C634G; D, diffuse; fMTC, familial MTC; ind, indeterminate cytology; L, linear; lfn, lymph nodes; MNG, uni/multinodular goiter; N, nodular; NA, paraffin blocks for the definition of the morphology and/or the count of C cells were not available; nd, cytology not done; neg, negative cytology; sMTC, sporadic MTC; susp, cytology suspicious for MTC; thy, associated thyroiditis; Tx, thyroidectomy.

^a The TNM classification and the pattern of CCH and the number of C cells per 10 fields are also reported.

Prevalence of MTC and CCH by preoperative CT level

The bCT levels ≤ 20 pg/mL were associated with MTC in 4 females (3 familial and 1 sporadic form) and in 3 males

(all familial forms). CCH was highly prevalent, both in women and men, even at low CT levels (≤ 20 pg/mL for bCT and ≤ 100 pg/mL for sCT). For bCT levels > 20 pg/mL, the prevalence of both CCH and MTC in women and

Table 2. Clinical and Histological Data of Male Patients (n = 42)^a

ID/Age, y	CT Pre-Tx, pg/mL		Histology (mm) ^{cytology}	TNM (Metastatic lfn/Total lfn)	CCH
	Basal	Peak			
bCT <10 pg/mL					
F.E./71	<2	2.9	PTC	pT3N0	No
C.G./24	2	4.3	PTC	pT4mN1bM1	No
O.L.M./43	<2	14	FA		No
T.S./49	<2	14.4	FA		D
F.O./55	2.4	18.5	FTC	pT3NX	No
B.R./60	<2	28.5	MNG		No
C.M./69	<2	40	MNG		NA
G.P.A./32	3.9	61.2	PTC	pT1NX	N
P.M./47	4.3	73.4	PTC	pT2mN0	No
B.M./8, #1	7.7	95.2	MNG		No
V.G./68	7.9	157	MNG		N
R.G./58	8.6	190	MNG		L (32.3)
R.U./60, #1	9.5	224	fMTC (5) nd	pT1N0 (0/6)	N
M.S./20	8.5	229	MNG		L, D, N (78.3)
bCT >10 pg/mL					
M.M./76	11.9	130	PTC	pT1NX	No
R.F./27, #1	17.2	135	fMTC (2) nd	pT1aN1a (1/4)	D
P.P./38	10.1	176	FTC	pT2NX	NA
D.P.P./65	12	196	MNG		L
I.D./58	13	226	PTC	pT1NX	L, D, N (86)
I.D./59	14	266	PTC	pT1bNX	No
S.M./44	22.1	278	PTC	pT1N0 (0/8)	L, D, N (71)
V.G.P./65	15.2	288	MNG		N
B.L./9, #2	13	296	fMTC (2) nd	pT1amN0 (0/3)	No
C.F./41	11.2	413	PTC ^{thy}	pT1NX	N
P.G./64	12.7	443	PTC	pT1NX	L, D, N (132)
N.V./72	154	467	sMTC (28) ^{susp}	pT3N1b (6/18)	No
S.C./42	15	480	MNG		L, D, N (87.7)
T.A./66	19.4	492	PTC (1)	pT1NX	L, D, N (94.7)
V.M./47	11.3	544	PTC ^{thy}	pT1NX	N
G.E./50	87.4	562	sMTC (7) nd	pT1aN0 (0/6)	No
M.D./65	31.4	589	MNG		L
S.E./81	72	800	sMTC (9) ^{nd, thy}	pT1NX	NA
C.M.A./54	32	1368	MNG		L, D, N (92.2)
S.F./67	312	1389	sMTC (13) ^{ind}	pT1N0 (0/10)	NA
B.E./72	90	1414	MTC (7) nd + PTC	pT1mNX/pT1mNX	NA
P.G./70	68	1620	MNG		N
D.C.L./59	437	2800	sMTC (14) ^{neg}	pT1N0 (0/20)	L, D, N (64.7)
B.G./65	198	2900	sMTC (17) nd	pT1bN0 (0/9)	No
Z.S./49	140	3687	sMTC (15) nd	pT1bN0 (0/3)	No
S.S./66	758	3890	sMTC (19) nd	pT1bN0 (0/9)	No
L.G./61	170	15700	sMTC (14) nd	pT1bNX	No
D.C./38	1860	20000	sMTC (25) nd	pT2N0 (0/22)	No

Abbreviations: #1, gene carrier *RET* S891A; #2, gene carrier *RET* C634Y; D, diffuse; fMTC, familial MTC; ind, indeterminate cytology; L, linear; lfn, lymph nodes; MNG, uni/multinodular goiter; N, nodular; NA, paraffin blocks for the definition of the morphology and/or the count of C cells were not available; nd, cytology not done; neg, negative cytology; sMTC, sporadic MTC; susp, cytology suspicious for MTC; thy, associated thyroiditis; Tx, thyroidectomy.

^a The TNM classification and the pattern of CCH and the number of C cells per 10 fields are also reported.

men paralleled the increasing CT levels in a gender-specific manner (Table 3). Interestingly, according to previous reports (10, 16), women had MTC at lower bCT levels (67% vs 0% for bCT levels ≤50 pg/mL and 100% vs 75% for bCT levels ≤100 pg/mL). The bCT levels >100 pg/mL were always associated with MTC in both females and males. Concerning sCT levels, the prevalence of MTC was higher in females for levels ≤500 pg/mL, whereas the prevalence was comparable for sCT levels >500 pg/mL (Table 3).

Sensitivity, specificity, positive and negative predictive value, and accuracy for CCH and MTC (ROC analysis)

ROC plot analyses were used to find the bCT and sCT thresholds able to differentiate between normal, CCH, and MTC for men and women. To increase the potency of the analysis, the patient data included in the present study have been pooled with the data obtained in our previous study (10). In females, bCT >26 pg/mL had the best ac-

Table 3. Prevalence of MTC and CCH by Preoperative bCT and sCT Levels in Female and Male Patients Who Underwent Total Thyroidectomy^a

CT pg/mL	Females (n = 49)			Males (n = 42)			Total (n = 91)		
	Total n	MTC, n (%)	CCH, n (%)	Total n	MTC, n (%)	CCH, n (%)	Total n	MTC, n (%)	CCH, n (%)
Basal									
≤10	21	1/21 (4.8)	3/16 (1.9)	14	1/14 (7.1)	6/13 (46.1)	35	2/35 (5.7)	9/29 (31)
≤20	38	4/38 (10.5)	12/29 (41.4)	27	3/27 (11.1)	15/25 (60)	65	7/65 (10.7)	27/54 (50)
>20	11	9 (82)	7/10 (70)	15	11 (73)	5/12 (42)	26	20 (77)	12/22 (55)
21–50	6	4 (67)	4/6 (67)	3	0	3/3 (100)	9	4 (44.4)	7/9 (77.7)
51–100	2	2 (100)	1/2 (50)	4	3 (75)	1/2 (50)	6	5 (83.3)	2/4 (50)
Peak									
>100	3	3 (100)	2/2 (100)	8	8 (100)	1/7 (14)	11	11 (100)	3/9 (33.3)
≤100	25	1 (4)	6/21 (29)	10	0	2/9 (22)	35	1 (2.8)	8/30 (26.6)
>100	22	13 (59)	14/20 (70)	32	14 (44)	18/28 (64)	54	27 (50)	32/48 (67)
101–500	12	6 (50)	6/11 (55)	18	4 (22)	13/17 (77)	30	10 (33.3)	19/28 (67.8)
501–1000	4	2 (50)	3/4 (75)	4	2 (50)	2/3 (75)	8	4 (50)	5/7 (71.4)
>1000	6	5 (83)	5/5 (100)	10	8 (80)	3/8 (38)	16	13 (81.2)	8/13 (61.5)

^a For CCH, the prevalence has been calculated considering patients for whom the data were available.

curacy to distinguish the non-MTC group (including patients without C-cell disease or with CCH) from the MTC group, whereas bCT >10 pg/mL differentiated between normal subjects and cases with CCH or MTC. In males, bCT >68 pg/mL was the threshold to discriminate non-MTC cases from MTC patients and >8 pg/mL the best

value to distinguish normal cases from patients with CCH or MTC (Figure 1). In females, a Ca sCT >79 pg/mL was able to distinguish non-MTC cases from patients with MTC (Figure 2). Ca sCT >55 pg/mL showed the best accuracy in the separation between normal subjects and patients with CCH or MTC. In males, a Ca sCT >544

Cut-offs for basal CT levels

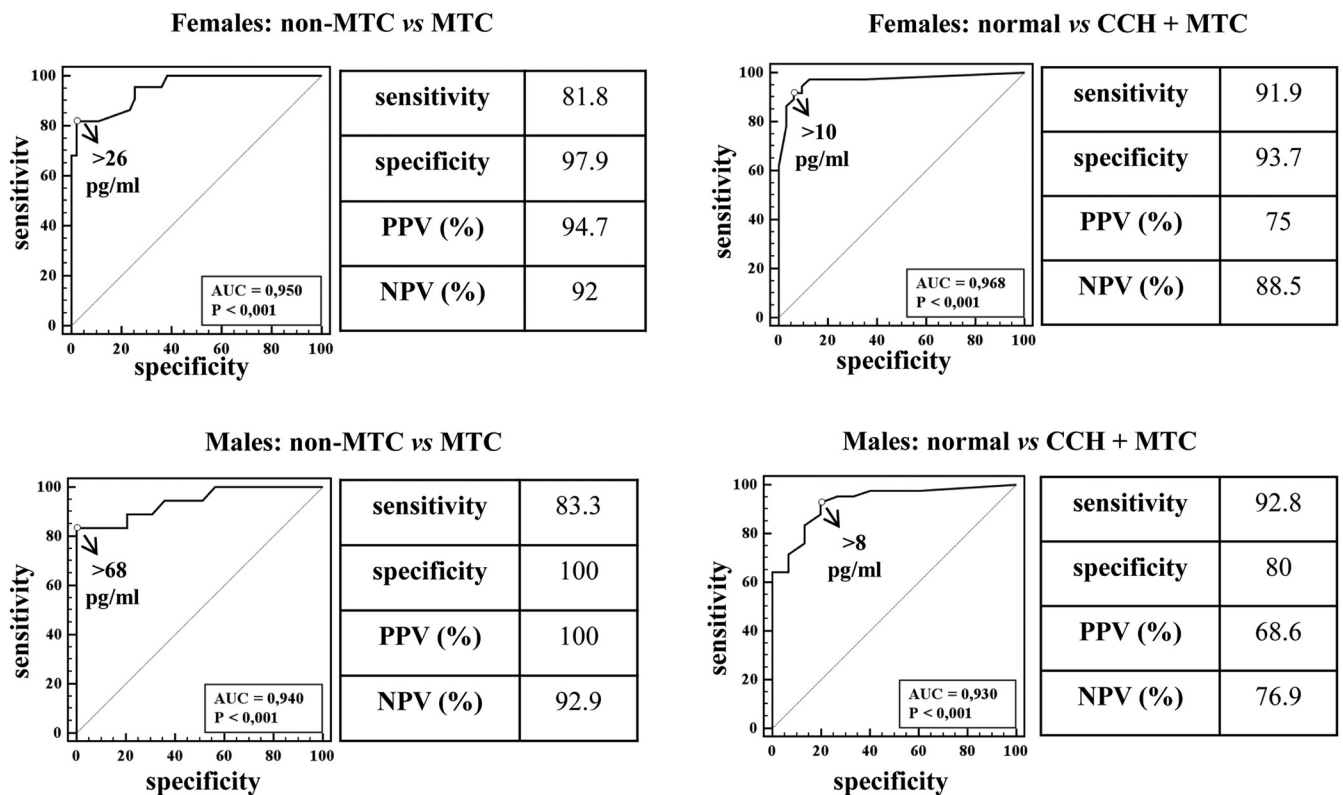


Figure 1. ROC curve analyses to identify the cutoff levels for bCT with the highest accuracy to differentiate between normal, CCH, and MTC in females (upper panel) and males (lower panel). Non-MTC represents normal thyroid and CCH. Abbreviations: NPV, negative predictive value; PPV, positive predictive value.

Cut-offs for Ca stimulated CT levels

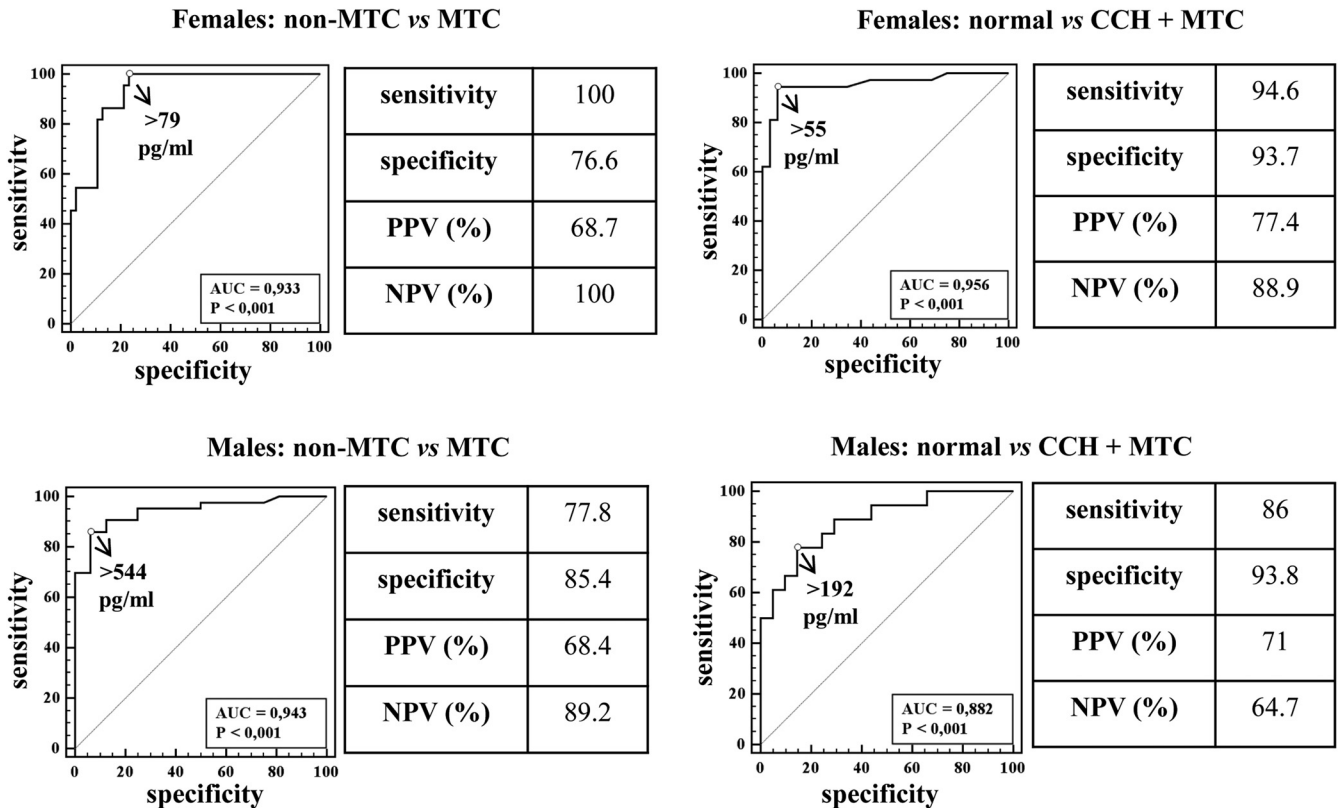


Figure 2. ROC curve analyses to identify the cutoff levels for sCT with the highest accuracy to differentiate between normal, CCH, and MTC in females (upper panel) and males (lower panel). Non-MTC represents normal thyroid and CCH. Abbreviations: NPV, negative predictive value; PPV, positive predictive value.

differentiated between non-MTC and MTC patients. On the other hand, to distinguish between normal subjects and cases with CCH or MTC, the best accuracy was shown by Ca sCT levels >192 pg/mL.

It is worth noting that the area under the ROC curve (AUC) for the differential diagnosis between non-MTC and MTC was very similar between bCT levels or sCT levels. In particular, the AUC was 0.950 for bCT and 0.933 for sCT in females and 0.940 for bCT and 0.943 for sCT in males. To distinguish between normal and CCH and MTC, the AUC was 0.968 for bCT and 0.956 for sCT in females and 0.930 for bCT and 0.882 for sCT in males (Figures 1 and 2).

The thresholds to distinguish between non-MTC and MTC patients did not vary after the exclusion of MTCs >10 mm (4 females and 8 males) that could have potentially been diagnosed by fine-needle aspiration cytology (>79 pg/mL, sensitivity 100%, specificity 76.6%, AUC 0.895 for females and >544 pg/mL, sensitivity 57.1%, specificity 85.4%, AUC 0.739 for males).

Finally, ROC analyses were also calculated after the exclusion of the patients with bCT levels <10 pg/mL. For both females and males, the cutoffs for bCT did not

change, whereas the cutoffs for sCT were higher and displayed a lower sensitivity and a higher specificity. In particular, the sCT cutoffs were >599 (AUC 0.79, sensitivity 57.1%, specificity 93.3%) in females and >1368 (AUC 0.80, sensitivity 61.1%, specificity 90.0%) in males.

Safety and side effects

No cardiac effects were observed either during or after Ca infusion. In particular, heart frequency variations were found at the ECG assessments performed during the test in only 1 of 91 patients, who experienced a transient bradycardia (40 beats/min). Moreover, in the 3 patients who needed an adjustment of the electrolyte levels before the test, no adverse effects were noted. No arrhythmias were recorded during ECG monitoring. In a male patient, the preinfusion evaluation revealed abnormal ECG findings suggestive of Brugada syndrome (Supplemental Figure 1A, published on The Endocrine Society’s Journals Online website at <http://jcem.endojournals.org>), and he thus underwent specific tests, including an echocardiogram, that allowed us to exclude the above mentioned cardiac disease. Therefore, the Ca test was performed without adverse effects. Moreover, at the pre- and posttest ECG, si-

nus rhythm, normal atrioventricular conduction, minor intraventricular conduction delay, and normal repolarization were recorded (Supplemental Figure 1, B and C). In the 10 patients who underwent tissue Doppler echocardiography, no changes in left and right ventricular systolic function were noted, whereas modifications in left diastolic ventricular function were recorded. In particular, during Ca administration, a significant increased ratio of mitral early blood inflow velocity to early diastolic velocity of the mitral annulus (E/E') was found, as a result of the inotropic action of calcium on heart muscle. Finally, no significant variations in blood pressure were recorded.

Discussion

The present study was aimed to find the most accurate bCT and Ca sCT cutoffs for the preoperative identification of subjects with CCH or MTC. The data obtained represent a refinement of those reported by our group in 2010 in a more limited number of cases (10). After pooling those results with the data obtained in this larger series of 91 patients, ROC curve analyses were used to compare the preoperative bCT and Ca sCT levels with the histological findings. The results showed that, for bCT, the cutoff points able to separate non-MTC (including normal and CCH cases) from MTC patients were >26 in females and >68 pg/mL in males, whereas the best Ca sCT thresholds for the identification of MTC were >79 and >544 pg/mL for women and men, respectively. The thresholds for bCT are comparable to those found in other reports (10, 16, 17), whereas sCT thresholds are lower than those found in our previous, more limited, series (>184 for females and >1620 pg/mL for males). These novel and accurate cutoffs are similar to those recommended for the gender-related evaluation of CT stimulated by Pg (18), suggesting that they are more reliable and definite than those previously reported and that can be safely introduced in clinical practice. Most data from the literature show that the positive predictive value of bCT in the diagnosis of MTC is lower than that of sCT (19). In contrast, in the present series, the AUC for bCT and sCT are similar, indicating that bCT values are at least as good predictors of MTC as are sCT levels and suggesting that serum CT assays with improved functional sensitivity may avoid the stimulation test in several conditions. The possibility of relying on only bCT would definitely increase the cost-effectiveness of the measurement of CT in the diagnosis of MTC. Nevertheless, the overall cost of the procedure in our departments (including the ECG monitoring, the pretest assessment of the electrolyte balance, the measurement of CT in the 3 samples after infusion, and the costs for the personnel in-

involved) has been calculated in around 82 euros per patient, which is significantly lower than that of the Pg test (around 196 euros, mainly due to the higher cost of Pg). As a general remark and thus not limited to the present series, we believe that the Ca test will maintain its relevance when one wants to exclude MTC in an unaffected individual with bCT in the gray zone, as in autoimmune thyroiditis or in neuroendocrine tumors, or attain a preclinical diagnosis in carriers of intermediate- or low-risk *RET* proto-oncogene mutations. Consistently, it has been recently shown that, in this latter condition, the ultrasensitive CT assay reduces the false-negative rate of bCT measurements, but its sensitivity to detect C-cell disease remains lower than that of sCT (20).

In most cases, a detailed evaluation of the C-cell component was done on histological samples. The overall prevalence of CCH in patients with bCT <10 pg/mL was 30% and rose to 39% and 68% for bCT ≤ 20 pg/mL in females and males, respectively. Accordingly, CCH was found to be frequent for sCT levels >100 pg/mL (70% and 64% in females and males, respectively). At ROC plot analysis, low cutoff levels were found for the identification of CCH (>55 in women and >192 pg/mL in men). As expected, CCH was almost invariably associated with MTC in familial cases. Interestingly, among cases subjected to Ca test for bCT ≥ 10 pg/mL, CCH was extremely frequent in MNGs and in PTCs but was seldom found in sporadic MTC cases. Moreover, according to our previous observations (16), CCH often displayed a diffuse and nodular distribution pattern, although it remains to be established whether this condition can be considered a carcinoma in situ of the parafollicular cells in *RET*-negative patients. The gender-related differences in both basal and stimulated CT levels, previously reported for both Pg and Ca tests, were confirmed in the present series. In particular, either sCT levels, or the Δ -increases, were significantly higher in males than in females ($P = .005$).

In the present large and multicentric series, the high tolerability of the Ca test was confirmed, with minor side effects mostly consisting of a brief feeling of warmth. Importantly, precise instructions to be followed before and during the test are provided: 1) to obtain anamnestic and pharmacological data; 2) to perform a pretest ECG evaluation; c) to measure pretest electrolyte levels; 4) to calculate the dose of calcium gluconate on the adjusted body weight (25 mg Ca gluconate/kg); 5) to infuse at 5 mL/min with a minimum time of administration of 3 minutes. Moreover, continuous cardiac monitoring should be done during the test to ensure a prompt intervention in the case of cardiac alterations, as recently reported in 1 male subject (21). Nevertheless, it must be emphasized that in 90 of 91 cases, no heart rate variations were documented. In a

particularly anxious patient, a bradycardia with a spontaneous resolution was observed, likely related to a vasovagal reaction. Hence, patients should be reassured about the safety of the test.

In conclusion, present data confirm that the high-dose Ca test is reliable, potent, and safe. The Ca sCT thresholds for the identification of CCH and MTC have been calculated and found to be similar to those used worldwide for the Pg test. Thus, approximately 3 years after its reintroduction in clinical practice, the Ca test has been demonstrated to harbor all the required features to replace the Pg test when the evaluation of an sCT is required.

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