Received : 04-Jan-2012

Returned for Revision: 12-Jun-2012

Finally Revised : 28-Jun-2012

Accepted : 27-Jul-2012

Article Type: B

Is basal ultrasensitive measurement of calcitonin capable of substituting for the pentagastrin-stimulation test?

Short title: Calcitonin measurement in medullary thyroid carcinoma

Géraldine Pina MD [1*], Séverine Dubois MD [2], Arnaud Murat MD, PhD [3], Nicole Berger MD, PhD [4], Patricia Niccoli MD, PhD [5], Jean-Louis Peix MD, PhD [4], Régis Cohen MD, PhD [6], Claudine Guillausseau MD, PhD [7], Anne Charrie MD [4], Olivier Chabre MD, PhD [8], Catherine Cornu PhD[1], Françoise Borson-Chazot MD, PhD [1**], Vincent Rohmer MD, PhD [2**]; on behalf of the Groupe des Tumeurs Endocrines

[1]Hospices Civils de Lyon et Université Lyon 1, Centre de Médecine Nucléaire, Centre d'Investigation Clinique et Fédération d'Endocrinologie, Groupement Hospitalier Est, Lyon, France

[2]CHU d'Angers, Département d'Endocrinologie Diabétologie Nutrition, Angers Cedex 09, F-49933 France

[3]CHU Nantes, Service d'Endocrinologie, Nantes, France

[4]Hospices Civils de Lyon, Services de Radioanalyse, Anatomo-pathologie et Chirurgie, Centre Hospitalier Lyon-Sud, Lyon, France

[5]APHM de Marseille, Service d'Endocrinologie, Hôpital de la Timone, Marseille, France

[6]APHP Paris, CHU 93, Service d'Endocrinologie, Hôpital Avicenne, Bobigny, France

[7]APHP Paris, Service de Médecine Nucléaire, La Pitié Salpétrière, Paris, France

[8]CHU de Grenoble, Service d'Endocrinologie, Grenoble, France

[*] Corresponding author

[**] Both are senior authors

Correspondance to:

Geraldine Pina, Centre de Médecine Nucléaire, Groupement Hospitalier Est, 59 boulevard Pinel, 69677 Bron Cedex, France.

Tel: + 33 (0) 4 72 35 72 77; Fax: + 33 (0) 4 72 35 73 45; Email: gpina.jomir@gmail.com

Keywords: second generation calcitonin assay; medullary thyroid carcinoma; pentagastrin-stimulation test; C-cell disease, C-cell hyperplasia

Conflict of Interest and financial disclosure

Nothing to declare

Abstract

Objective. To evaluate a second-generation assay for basal serum calcitonin measurements compared to the pentagastrin-stimulation test for the diagnosis of inherited medullary thyroid carcinoma (MTC) and the follow-up of MTC patients after surgery. Recent American Thyroid Association recommendations suggest the use of basal calcitonin (CT) alone to diagnose and assess follow-up of MTC as the pentagastrin (Pg) test is unavailable in many countries.

Design. Multicentric prospective study

Patients. A total of 162 patients with basal CT <10 ng/L were included: 54 asymptomatic patients harboured non-cysteine 'rearranged during transfection' (*RET*) proto-oncogene mutations and 108 patients had entered follow-up of MTC after surgery.

Measurement. All patients underwent basal and Pg-stimulated calcitonin measurements using a second-generation assay with 5-ng/L functional sensitivity.

Results. 95% of patients with basal CT ≥5 ng/L and 25% of patients with basal CT <5 ng/L had a positive Pg-stimulation test (Pg CT >10 ng/L). Compared to the reference Pg test, basal CT ≥5 ng/L had 99% specificity, a 95%-positive predictive value but only 35% sensitivity (p <0.0001). Overall, there were 31% less false negative results using a 5-ng/L threshold for basal CT instead of the previously used 10-ng/L threshold.

Conclusion. The ultrasensitive calcitonin assay reduces the false negative rate of basal calcitonin measurements when diagnosing familial MTC and in postoperative follow-up compared to previously used assays. However, its sensitivity to detect C-cell disease remains lower than that of the Pg-stimulation test.

Introduction

Calcitonin (CT) is a sensitive and specific marker for the pre-operative diagnosis of C-cell disease and to monitor medullary thyroid carcinoma (MTC) patients after surgery [1, 2]. In the pre-operative state, the positive predictive value (PPV) of basal CT to diagnose MTC is nearly 100% when CT is >100 ng/L, but is only 8.3% for a CT level between 20 and 50 ng/L[3]. CT stimulation with pentagastrin (Pg) has been shown to be more sensitive than basal CT to differentiate between microscopic MTC and non-specific elevated CT in patients with moderately elevated CT levels [4-6]. It has also been used to improve sensitivity of basal CT screening in genetically predisposed patients and for the postoperative follow-up of MTC patients.

In genetically predisposed patients with intermediate or low-risk 'rearranged during transfection' (*RET)* proto-oncogene mutations, for whom prophylactic surgery is postponed until after early infancy [7, 8], C-cell disease may be already present even though basal CT is within the normal range (<10 ng/L). The chosen threshold used to assess a positive Pg response for this indication is usually 10 ng/L for the lowest false-negative rate possible [7, 8]. However, recent retrospective studies suggest that the timing of thyroidectomy can be probably safely planned based on basal CT levels [9, 10].

In the postoperative follow-up of patients with MTC, basal CT measurements allow detection of residual disease whereas the Pg-stimulation test is more sensitive at diagnosing residual disease or its recurrence in patients with low serum CT values [2, 5, 8, 11, 12]. Patients who have non-detectable and non-stimulable postoperative CT levels at two consecutive follow-up visits are considered disease-free. They still require yearly follow-up assessments as there can be a ≤5% late recurrence of disease [13, 14]. However, the consequences of a positive stimulation test on clinical management and long-term outcomes have not been demonstrated [3].

As Pg is not available in the United States and many other countries [15] and because unpleasant adverse effects of Pg are very frequent and restrain its use, an alternative test using calcium

stimulation (Ca gluconate injection) has been proposed and shows better clinical tolerance[16]. Results of both stimulation tests have been reported to be well correlated [17].

The functional sensitivity of the previously used CT assay was 10 ng/L [7, 8, 18]. However, second-generation CT assays with a functional sensitivity of <5 ng/L are now available and are widely used. Because the newer calcitonin assays have significantly improved functional sensitivity, the recent American Thyroid Association (ATA) guidelines stated that "most experts believe that there is rarely a need for stimulated CT testing in the diagnosis or follow-up of MTC" [19].

In this context, this study evaluated the performances of second-generation baseline CT measurements compared to Pg-stimulated CT to diagnose pre-operative inherited intermediate or low risk C-cell disease and to assess the postoperative follow-up of patients with sporadic or familial MTC. Comparisons with histopathology (when available) were also conducted.

Subjects and methods

Subjects

This prospective, multicentre study included 162 patients. Patients were recruited from seven university hospital centres in France (Angers, Lyon, Marseille, Nantes, Paris la Pitié Salpétrière, Paris Bobigny, Grenoble) and were divided into two groups. Inclusion criteria for group 1 were intermediate or low risk *RET* mutation (level A or B), no history of thyroidectomy and a baseline CT level of ≤10 ng/L. For group 2 they were surgery for MTC (sporadic or inherited, whatever the risk level), carcinoembryonic antigen (CAE) level of ≤10 ng/L and baseline CT level of ≤10 ng/L. Group 1 contained 54 asymptomatic patients harbouring *RET* mutations at exons 13, 14 and 15 (codons 768, *n*=3; 790, *n*=24; 891, *n*=10; 804. *n*=14) or double mutations of exon 14 (804–844; *n*=3). The results from five patients have been reported in a recent publication [10].

Group 2 included 108 patients with a history of sporadic or familial MTC, who had undergone surgery and showed no clinical signs of metastasis or recurrence of MTC. Mean age and gender ratio for groups 1 and 2 are listed in Table 1. Among group 1 patients, 40 had undergone surgery by the time results were collected. The decision to perform surgery took into account the type of mutation, the patient's family history and the results of the Pg-stimulation test.

Serum calcitonin assay and pentagastrin test

Serum CT levels (for basal determination and after Pg stimulation) were measured using a second-generation solid-phase, two-site, immunoradiometric assay (IRMA-hCT®; IBA CIS Bio International, Gif-Sur-Yvette, France), which is the most commonly used in France. Its analytical sensitivity was 1.5 ng/L. The functional sensitivity, the lowest concentration which gave a coefficient of variation of <20% in interassay variations, was 5 ng/L. All serum CT levels below functional sensitivity (<5 ng/L) were considered to be equal to 4.9 ng/L in this study. For the Pg-stimulation test, blood was collected before and at 0, 5, 15 and 30 min after intravenous Pg injection (0.5 g/kg). The Pg-stimulation test was performed before surgery in patients with germline *RET* mutations and after surgery during the follow-up of MTC patients.

Assessment criteria

All patients underwent baseline and Pg-stimulated serum CT measurements. The usual threshold of 10 ng/L was used for Pg-stimulated CT: a Pg-stimulated CT ≥10ng/L was considered pathological and the Pg-stimulation test was considered positive [8, 20, 21]. This 10-ng/L threshold corresponded to the functional sensitivity of previously used CT assays and was considered the most sensitive threshold when detecting disease at its earliest stage in *RET*-gene carriers and when assessing a biological cure in the post-operative follow-up period of MTC patients [7, 8, 22].

The results from baseline CT determinations were compared with those of the Pg tests in terms of sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV). The false-negative rate was calculated as the number of false-negative results divided by the number of false-negative plus true-negative results of basal CT measurements to predict a positive stimulation test. The multiplication factor between basal and stimulated CT level was calculated as the highest CT value after stimulation divided by the basal CT value.

For group 1 patients who underwent later surgery, the results of pre-operative baseline and Pg-stimulated CT determinations were also compared with their histopathological findings.

The sensitivity and specificity of basal CT, measured with the second-generation CT assay that diagnosed C-cell disease, were evaluated with a 5-ng/L threshold.

Diagnosis of MTC was based on typical histological characteristics and immunohistochemical findings (positive staining for CT and chromogranin, negative staining for thyroglobulin). C-cell hyperplasia (CCH) was diagnosed when at least three low-power fields containing ≥50 C-cells were present, as described by Guyetant et al. [23]. Serial sections (3 mm thick) were used for routine staining and immunohistochemistry.

Statistical analyses

Results are given as their means (M) ± standard deviations (SD). Sensitivity, specificity, PPV and NPV were calculated. Results were considered significant when chi-square values were >3.84 (p <0.05).

Results

Basal and Pg-stimulated CT

The results of basal and Pg-stimulated CT measurements performed in the 162 patients are presented in Table 1 and Figure 1. Results for *RET* mutations in group 1 patients are presented in Table 2.

Comparison between basal CT and the Pg-stimulation test

As shown in Figure 2, the Pg test was positive in 95% of patients with basal CT ≥5 ng/L. Only one of the 20 patients with basal CT ≥5 ng/L did not respond to the Pg test. In this patient, basal CT was 7 ng/L. For patients with basal CT <5 ng/L, a positive Pg test was obtained in 36 out of 142 patients (25%). Compared to the reference Pg test, basal CT of ≥5 ng/L had a 99% specificity, a 95% PPV but only 35% sensitivity (p <0.0001) (Table 3). In the pre-operative assessment of *RET* mutation carriers and for the postoperative follow-up after thyroidectomy, basal CT level predicted a positive Pg-stimulation response with 100% and 99% specificity, 100% and 86% PPV, and 39% and 27% sensitivity, respectively (p <0.001 for both).

Overall, the 5-ng/L threshold, instead of the previously used 10-ng/L threshold, reduced the false negative rate of basal CT: there were 36 false-negative results for a 5-ng/L threshold versus 55 false-negative results for a 10-ng/L threshold (31% reduction).

Comparison between pre-operative basal CT and Pg-stimulated CT with histopathology

Forty patients from group 1 underwent surgery. Histological examination showed microscopic MTCs in 16 patients, CCHs in 22 patients and no C-cell disease in 2 patients. Neither lymph-node metastasis

nor macroscopic MTC was found in any patient. All patients had non-detectable post-operative basal CT.

Among these 40 patients, post-operative histology showed CCH or MTC in all patients with basal CT <5 ng/L (*n*=28, 11 microscopic MTCs, 17 CCHs); 64% (18/28) had

a positive Pg-stimulation test. All patients (*n*=12) with a basal CT ≥5 ng/L (5–10 ng/L) had a positive Pg-stimulation test and 83% (10/12) showed CCHs or MTCs on postoperative histological examination.

Basal CT had a sensitivity of 29% and a 85% PPV value to detect C-cell disease (p <0.05); the number of false positives to detect MTC was 16 (Table 4). Pg-stimulated CT had a sensitivity of 71% and a 93% PPV to detect C-cell disease (Table 4). A false-negative result was observed in two female patients with microscopic MTCs and non-detectable non-stimulatable CT. One was aged 26 years, with a 804-844 mutation, a basal CT of 4.9ng/L and a stimulated CT of 6.2 ng/L. The second was aged 29 years, had a 790 mutation, a basal CT of 4.9 ng/L and a stimulated CT of 7.9 ng/L.

The mean multiplication factor (+ SD) between basal and Pg-stimulated CT was 4 + 0.8 (range: 3–5) in patients without C-cell disease, 3 + 2.0 (range: 1–8) in patients with HCC and 8 + 7.4 (range: 1–19) in patients with MTC.

Discussion

In this prospective study, we investigated the effectiveness of second-generation basal CT determination to diagnose pre-operative inherited C-cell disease (with intermediate or low risk) and for the post-operative follow-up of patients with sporadic or familial MTC. We found that second-generation basal CT was more sensitive than conventional basal CT but still remained less sensitive than Pg stimulation. In patients with basal CT ≥5 ng/L, a positive Pg test is expected and the usual

strategy is applied accordingly. In contrast, **the NPV of basal CT<5ng/L is lower than that of Pg stimulation test and a negative response to the Pg stimulation test cannot be assumed.**

The Pg-stimulation test is considered a reference for the diagnosis and follow-up of MTC [3, 4] because basal CT has several drawbacks. Reference ranges for CT levels in a healthy population have to be adapted to age and sex [18, 24]. False negative results have been reported in rare cases of non-CT-secreting MTCs [25, 26]. False-positive results can be observed in pathological conditions (hypercalcemia, hypergastrinemia, neuroendocrine tumours, renal insufficiency, goitre) and non-pathological circumstances (prolonged treatment with proton pump blockers like omeprazole, beta blockers, glucocorticoids, potential secretagogues like glucagon…), which decrease its specificity [18, 24, 27]. Thus, the PPV of basal CT is very low for moderately elevated CT level whereas Pg-stimulated CT confers a gain in specificity: the increase in CT level after stimulation is usually 5–10 times higher than basal CT in MTC whereas it is only moderately elevated in patients with other types of neuroendocrine tumours [28]. Furthermore, Pg-stimulated CT can be used to distinguish CCH from MTC [29]. **CT stimulation testing can be used for the screening of RET mutation carriers for family members who refuse DNA analysis [30] and has a prognostic value in MTC [31].**

As Pg is not always available, an alternative test using Ca stimulation has been proposed and shows significant correlation to Pg-stimulated CT, improved clinical tolerance **and may also be used to distinguish CCH from MTC for moderately elevated basal CT levels** [16, 17]. Nevertheless the American Thyroid Association MTC guidelines suggest that "newer calcitonin assays with improved functional sensitivities, should improve the performances of basal CT determination and limit the need for stimulated CT testing in the diagnosis and follow-up of MTC"[19]. However, the efficacy of second-generation basal CT determination alone has not been compared with stimulated CT tests. Thus, it is important to ascertain if the use of second-generation CT measurement improves the accuracy of basal CT measurements and whether the results are concordant with those from stimulation testing.

This study focused on two well-characterized situations: pre-operative diagnosis of inherited forms of C-cell disease (with intermediate or low risk) and post-operative follow-up of MTC (sporadic or familial with all risk levels).

Inherited medullary thyroid carcinomas show a good genotype–phenotype correlation. Thus, conventional strategy was, first, to perform prophylactic thyroidectomy on asymptomatic RET mutation carriers i.e. to operate before the occurrence of MTC. To achieve this goal, surgery should be performed during infancy, before the age of the earliest occurrence of MTC for the corresponding genotype [3, 19, 32, 33]. For low or intermediate risk RET mutation, an integrated DNA-based/biochemical concept has been proposed and offers more flexibility and the opportunity to postpone surgery at a stage when C-cell disease has already appeared. For instance, in the setting of normal annual neck ultrasonography, less aggressive MTC family history and family preference, thyroidectomy may be postponed and monitored on basal and stimulated-CT determination on a yearly basis. Because previously used CT assays lack sensitivity in the lowest ranges the Pg test has been recommended as the most sensitive test to detect C-cell disease at its earliest stage [7, 8, 33]. However, non-detectable non-stimulable pre-operative CT cannot guarantee prophylactic thyroidectomy, which is confirmed in our study by the significant number of false-negative Pg-stimulation tests in patients with microscopic MTCs. Therefore, despite better sensitivity, the clinical usefulness of Pg stimulated CT in terms of **practical interest for the patient is a matter of debate since** thyroidectomy performed at an early stage i.e. before the occurrence of lymph-node invasion results in an almost certain cure [8, 10, 19, 32-34] . In our study, no patients with basal CT between 5 and 10 ng/L had N1 or macroscopic MTCs, whatever the results of Pg stimulated CT. Accordingly, recent retrospective studies showed that moderately elevated pre-operative basal CT was always associated with a N0 status [9, 10]. Thus, the timing of surgery could be monitored using basal CT with no difference in long-term outcomes compared to monitoring based on stimulated CT. **The interest and extensiveness of lymph-node dissection is currently under investigation. In patients with low basal CT, Pg stimulation test was considered the most sensitive tool to identify the need for lymph node dissection. Recent studies**

suggested however that patients with normal or moderately elevated basal CT could forgo lymph node dissection as no lymph-node metastases are expected [9, 10, 35].

In the post operative follow-up period of MTC patients, a 10-year survival rate of 97.7% has been reported in biologically cured patients (defined as undetectable basal and stimulated CT) versus 70.3% for non-cured patients (subsequent elevation of basal or stimulated CT after post-operative normalisation), with a reported 5% late recurrence rate [13, 14]. The main limitation of the present study, which included 108 patients with post-operative basal CT levels of <10 ng/L, is the absence of long-term clinical and biological follow-ups to assess any recurrence. Thus, the Pg stimulation test was used as reference for assessing a biological cure. From our results, MTC patients after surgery with detectable basal CT (≥ 5 ng/L) should be considered as non cured and patients with undetectable basal CT (<5 ng/L) enter into long term follow-up. However, those patients do not benefit in the results of the Pg stimulation test apart from refining their prognostic assessment as there is no change in term of clinical management and no proven benefit in term of outcome: even though the Pg test remains the most sensitive tool to assess a biochemical cure [11, 12, 14], when only the stimulated CT level is detectable, the volume of residual disease is very low, unlikely to be found by imaging **until basal CT is over 150ng/L**, and the patient may enter into long-term follow-up [3] . As there is no change in the clinical management the usefulness of systematic postoperative Pg testing has been questioned by some authors[19].

In conclusion, second-generation basal CT has better sensitivity than previously used CT assays. However, its sensitivity remains lower than that of the Pg-stimulation test. Nevertheless, for the pre-operative screening of low or intermediate risk *RET* mutations in patients with familial MTC, basal CT alone is well tolerated, sensitive enough to detect disease at an early state and to select subjects for curative surgery before the occurrence of macroscopic MTC or lymph-node involvement. For MTC patients after surgery, low levels of residual disease may be present despite undetectable basal CT

values. As this minimal disease is unlikely to be localized or treated, basal CT alone is an appropriate tool to use during the postoperative follow-up.

Acknowledgements

We thank Drs Bernard Masson and Genevieve Barbe for their participation in the study, Pr Jacques Orgiazzi for critical lecture of the manuscript and stimulating discussion.

References

1 Cohen, R., Campos, J.M., Salaun, C., et al. (2000) Preoperative calcitonin levels are predictive of tumor size and postoperative calcitonin normalization in medullary thyroid carcinoma. Groupe d'Etudes des Tumeurs a Calcitonine (GETC). *J Clin Endocrinol Metab* **85**, 919-922.

2 de Groot, J., Kema, I., Breukelman, H., et al. (2006) Biochemical markers in the follow-up of medullary thyroid cancer. *Thyroid* **16**, 1163-1170.

3 Costante, G., Durante, C., Francis, Z., et al. (2009) Determination of calcitonin levels in C-cell disease: clinical interest and potential pitfalls. *Nat Clin Pract Endocrinol Metab* **5**, 35-44.

4 Costante, G., Meringolo, D., Durante, C., et al. (2007) Predictive value of serum calcitonin levels for preoperative diagnosis of medullary thyroid carcinoma in a cohort of 5817 consecutive patients with thyroid nodules. *J Clin Endocrinol Metab* **92**, 450-455.

5 Pacini, F., Schlumberger, M., Dralle, H., et al. (2006) European consensus for the management of patients with differentiated thyroid carcinoma of the follicular epithelium. *Eur J Endocrinol* **154**, 787-803.

6 Vierhapper, H., Bieglmayer, C., Kaserer, K., et al. (2005) Early diagnosis and curative therapy of medullary thyroid carcinoma by routine measurement of serum calcitonin in patients thyroid disorders. *Thyroid* **15**, 1267-1272.

7 Dralle, H., Gimm, O., Simon, D., et al. (1998) Prophylactic thyroidectomy in 75 children and adolescents with hereditary medullary thyroid carcinoma: German and Austrian experience. *World J Surg* **22**, 744-750; discussion 750-741.

8 Niccoli-Sire, P., Murat, A., Rohmer, V., et al. (2001) Familial medullary thyroid carcinoma with noncysteine ret mutations: phenotype-genotype relationship in a large series of patients. *J Clin Endocrinol Metab* **86**, 3746-3753.

9 Elisei, R., Romei, C., Renzini, G., et al. (2012) The Timing of Total Thyroidectomy in RET Gene Mutation Carriers Could Be Personalized and Safely Planned on the Basis of Serum Calcitonin: 18 Years Experience at One Single Center. *J Clin Endocrinol Metab* **97**, 426-435.

10 Rohmer, V., Vidal-Trecan, G., Bourdelot, A., et al. (2011) Prognostic factors of disease-free survival after thyroidectomy in 170 young patients with a RET germline mutation: a multicenter study of the Groupe Francais d'Etude des Tumeurs Endocrines. *J Clin Endocrinol Metab* **96**, E509-518.

11 Engelbach, M., Gorges, R., Forst, T., et al. (2000) Improved diagnostic methods in the follow-up of medullary thyroid carcinoma by highly specific calcitonin measurements. *J Clin Endocrinol Metab* **85**, 1890-1894.

12 Lips, C.J., Hoppener, J.W. and Thijssen, J.H. (2001) Medullary thyroid carcinoma: role of genetic testing and calcitonin measurement. *Ann Clin Biochem* **38**, 168-179.

13 Kebebew, E., Ituarte, P.H., Siperstein, A.E., et al. (2000) Medullary thyroid carcinoma: clinical characteristics, treatment, prognostic factors, and a comparison of staging systems. *Cancer* **88**, 1139-1148.

14 Modigliani, E., Cohen, R., Campos, J.M., et al. (1998) Prognostic factors for survival and for biochemical cure in medullary thyroid carcinoma: results in 899 patients. The GETC Study Group. Groupe d'etude des tumeurs a calcitonine. *Clin Endocrinol (Oxf)* **48**, 265-273.

15 Cooper, D.S., Doherty, G.M., Haugen, B.R., et al. (2009) Revised American Thyroid Association management guidelines for patients with thyroid nodules and differentiated thyroid cancer. *Thyroid* **19**, 1167-1214.

16 Doyle, P., Düren, C., Nerlich, K., et al. (2009) Potency and tolerance of calcitonin stimulation with high dose calcium versus pentagastrin in normal adults. *J Clin Endocrinol Metab* **94**, 2970-2974.

17 Colombo, C., Verga, U., Mian, C., et al. (2012) Comparison of calcium and pentagastrin tests for the diagnosis and follow-up of medullary thyroid cancer. *J Clin Endocrinol Metab* **97**, 905-913.

18 Basuyau, J.P., Mallet, E., Leroy, M., et al. (2004) Reference intervals for serum calcitonin in men, women, and children. *Clin Chem* **50**, 1828-1830.

19 Kloos, R.T., Eng, C., Evans, D.B., et al. (2009) Medullary thyroid cancer: management guidelines of the American Thyroid Association. *Thyroid* **19**, 565-612.

20 Niccoli, P., Wion-Barbot, N., Caron, P., et al. (1997) Interest of routine measurement of serum calcitonin: study in a large series of thyroidectomized patients. The French Medullary Study Group. *J Clin Endocrinol Metab* **82**, 338-341.

21 Pacini, F., Castagna, M.G., Brilli, L., et al. (2008) Differentiated thyroid cancer: ESMO clinical recommendations for diagnosis, treatment and follow-up. *Ann Oncol* **19 Suppl 2**, ii99-101.

22 Barbot, N., Calmettes, C., Schuffenecker, I., et al. (1994) Pentagastrin stimulation test and early diagnosis of medullary thyroid carcinoma using an immunoradiometric assay of calcitonin: comparison with genetic screening in hereditary medullary thyroid carcinoma. *J Clin Endocrinol Metab* **78**, 114-120.

23 Guyetant, S., Wion-Barbot, N., Rousselet, M.C., et al. (1994) C-cell hyperplasia associated with chronic lymphocytic thyroiditis: a retrospective quantitative study of 112 cases. *Hum Pathol* **25**, 514-521.

24 d'Herbomez, M., Caron, P., Bauters, C., et al. (2007) Reference range of serum calcitonin levels in humans: influence of calcitonin assays, sex, age, and cigarette smoking. *Eur J Endocrinol* **157**, 749-755.

25 Dora, J.M., Canalli, M.H., Capp, C., et al. (2008) Normal perioperative serum calcitonin levels in patients with advanced medullary thyroid carcinoma: case report and review of the literature. *Thyroid* **18**, 895-899.

26 Wang, T.S., Ocal, I.T., Sosa, J.A., et al. (2008) Medullary thyroid carcinoma without marked elevation of calcitonin: a diagnostic and surveillance dilemma. *Thyroid* **18**, 889-894.

27 Toledo, S.P., Lourenco, D.M., Jr., Santos, M.A., et al. (2009) Hypercalcitoninemia is not pathognomonic of medullary thyroid carcinoma. *Clinics (Sao Paulo)* **64**, 699-706.

28 Baloch, Z., Carayon, P., Conte-Devolx, B., et al. (2003) Laboratory medicine practice guidelines. Laboratory support for the diagnosis and monitoring of thyroid disease. *Thyroid* **13**, 3-126.

29 Milone, F., Ramundo, V., Chiofalo, M.G., et al. (2011) Predictive value of pentagastrin test for preoperative differential diagnosis between C-cell hyperplasia and medullary thyroid carcinoma in patients with moderately elevated basal calcitonin levels. *Clin Endocrinol (Oxf)* **73**, 85-88.

30 Pacini, F., Castagna, M.G., Cipri, C., et al. (2010) Medullary thyroid carcinoma. *Clin Oncol (R Coll Radiol)* **22**, 475-485.

31 Machens, A., Hauptmann, S. and Dralle, H. (2008) Medullary thyroid cancer responsiveness to pentagastrin stimulation: an early surrogate parameter of tumor dissemination? *J Clin Endocrinol Metab* **93**, 2234-2238.

32 Machens, A. and Dralle, H. (2007) Genotype-phenotype based surgical concept of hereditary medullary thyroid carcinoma. *World J Surg* **31**, 957-968.

33 Machens, A. and Dralle, H. (2009) Prophylactic thyroidectomy in RET carriers at risk for hereditary medullary thyroid cancer. *Thyroid* **19**, 551-554.

34 Ball, D.W. (2009) American Thyroid Association guidelines for management of medullary thyroid cancer: an adult endocrinology perspective. *Thyroid* **19**, 547-550.

35 Machens, A., Lorenz, K. and Dralle, H. (2009) Individualization of lymph node dissection in RET (rearranged during transfection) carriers at risk for medullary thyroid cancer: value of pretherapeutic calcitonin levels. *Ann Surg* **250**, 305-310.

Figure legends

Figure 1: Scatterplot of basal and Pg-stimulated CT.

CT: calcitonin; Pg: pentagastrin

Figure 2: Results of Pg-stimulation test for 'negative' level of basal CT (<5 ng/L) and for 'positive' level of basal CT (≥5 ng/L) in all patients (*n* =162), in the pre-operative assessment of gene carriers (group 1, *n* =54) and in the postoperative follow-up of MTC patients (group 2, *n* = 108). The Pg-stimulation test was considered positive when Pg-stimulated CT was >10 ng/L. Basal CT measurement was considered positive when basal CT was >5 ng/L

CT: calcitonin; Pg: pentagastrin; MTC: medullary thyroid carcinoma

Table legends

Table 1:

Abbreviations: F: female, M: male, SD: standard deviation, CT: calcitonin, Pg: pentagastrin, MTC: medullary thyroid carcinoma, *n*: number.

Table 2:

Abbreviations: Pg: pentagastrin; CT: calcitonin; No: number, F: female; M: male; SD: standard deviation

Table 3:

Basal CT measurement was considered positive when basal CT was ≥5 ng/L. The Pg-stimulation test was considered positive when Pg-stimulated CT was ≥10 ng/L.

Abbreviations: CT: calcitonin; Pg: pentagastrin; PPV: positive predictive value; NPV: negative predictive value.

Table 4:

Basal CT measurement was considered positive when ≥5 ng/L. The Pg-stimulation test was considered positive when Pg-stimulated CT was ≥10 ng/L.

Abbreviations: CT: calcitonin; NPV: negative predictive value; Pg: pentagastrin; PPV: positive predictive value

Table 1: Characteristics of patients, basal and Pg stimulated CT measurement in all patients (n =**162), Group 1 (pre-operative assessment in gene carriers;** n =**54) and Group 2 (post operative follow-up of MTC patients;** n =**108).**

	All patients	**Group 1**	**Group 2**
Number of patients	162	54	108
Sex, F/M	89/73	24/30	65/43
Age (years) : mean± SD	21.5±15.9	24.3±16.6	48.5±16.3
(range)	(3–82)	(3–73)	(6–82)
Basal CT (ng/L)	5.1±0.8	5.38±1.2	5.0±0.6
(range)	(4.9–9.9)	(4.9–9.9)	(4.9–8.3)
Number of patients with basal CT > 5	20	13	7
Pg-stimulated CT	19.6±31.7	26.8±36.6	16.1±28.4
(range)	(4.9–179.0)	(4.9–179.0)	(4.9–145)

Legend Table 1:

F: female, M: male, SD: standard deviation, CT: calcitonin, Pg: pentagastrin, MTC: medullary thyroid carcinoma, n: number.

Table 2: *RET* mutation and results of basal and Pg stimulated CT measurement in Group 1 patients (n=54)

	***RET* mutation**				
	790	**891**	**804**	**804–844**	**768**
No. of patients	24	10	14	3	3
Sex, F/M	10/14	5/5	3/11	3/0	3/0
Age : mean ± SD	21.5±15.9	23.4±22.4	28.0±17.1	29.7±4.7	27.7±1.5
(range)	(4–68)	(6–73)	(3–54)	(26–35)	(26–29)
No. of patients with Basal CT ≥5ng/L	7	5	1	0	0
No. of patients with Pg CT ≥10ng/L	14	8	7	1	3
Basal CT:					
mean ± SD	5.5±1.3	6.0±1.6	4.9±0.0	4.9	4.9
(range)	(4.9–9.9)	(4.9–9.0)	(4.9–5.0)		
Pg-stimulated CT					
mean ± SD	21.7±30.7	47.9±53.3	14.3±13.5	9.7±7.2	71.9±59.8
(range)	(4.9–133.0)	(4.9–179.0)	(4.9–52.2)	(4.9–18.0)	(37.4–141.0)

Legend Table 2:

Pg: pentagastrin; CT: calcitonin; No: number, F: female; M: male; SD: standard deviation

Table 3: Sensitivity, specificity, PPV and NPV of basal CT measurement versus Pg stimulation test for all patients (N=162), pre-operative assessment of gene carriers (Group 1, N=54) and post operative follow-up of MTC patients (Group 2, N=108).

	All patients N=162	Group 1 N=54	Group 2 N=108
Sensitivity %	35	39	27
Specificity %	99	100	99
PPV %	95	100	86
NPV %	75	51	84
p	<0.0001	<0.001	<0.0001

Legend Table 3:

Basal CT measurement was considered positive when basal CT was superior or equal to 5ng/L. The Pg stimulation test was considered positive when Pg stimulated CT was superior or equal to 10ng/L.

CT: calcitonin; Pg: pentagastrin; PPV: positive predictive value; NPV: negative predictive value.

Table 4: Sensitivity, specificity, PPV and NPV of pre-operative basal CT measurement and Pg stimulation test for Group 1 patients who underwent thyroidectomy (N=40) for the detection of C-Cell disease

Detection of C-Cell disease	Basal CT	Pg CT
Sensitivity %	29%	71%
Specificity %	0%	0%
PPV %	85%	93%
NPV %	0%	0%
p	<0.05	

Legend table 4:

Basal CT measurement was considered positive when superior or equal to 5ng/L. The Pg stimulation test was considered positive when Pg stimulated CT was superior or equal to 10ng/L.

CT: calcitonin; NPV: negative predictive value; Pg: pentagastrin; PPV: positive predictive value