Calcitonin screening and pentagastrin testing: predictive value for diagnosis medullary carcinoma in nodular thyroid disease

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\textbf{short title:} Herrmann BL et al., calcitonin screening in nodular thyroid disease

\textbf{keywords:} calcitonin screening, pentagastrin testing, medullary carcinoma

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Summary

Context: Serum calcitonin (hCT) measurement may be useful for detecting medullary carcinoma (MTC), but the routine use of hCT and after pentagastrin stimulation to screen patients with nodular thyroid disease remains controversial.

Patients: 1007 patients (667 females, 440 males) with nodular thyroid disease and a mean age of 55±14 (mean±SD) years were included in the study. All patients did not have impaired renal function, bacterial infection, alcohol- and drug-abuse, pseudohypoparathyroidism, or proton pump inhibitor therapy. Individuals referred with known elevation of hCT, Graves’ disease or autoimmune thyroid disease were not considered or included to be part of this investigation.

Methods: Serum hCT levels were determined under basal conditions, and when basal values were more than or equal to 10 and less than 100 pg/ml, testing was repeated after pentagastrin stimulation. Basal or stimulated levels more than 100 pg/ml were indicated for surgery.

Results: hCT levels >10 pg/ml were increased in 17 patients (1.7%). One patient had a basal hCT level of 4400 pg/ml with a histological confirmation of a MTC. In this patient, pentagastrin test was not performed. 16 patients with basal hCT between 10-100 pg/ml underwent pentagastrin-stimulated hCT measurement. 4/16 had stimulated hCT >100 pg/ml. 2/17 patients with hCT >10 pg/ml had MTC and 3/17 patients C cell hyperplasia. In sum, 2 patients (0.20%) had histological verified MTC.

Conclusions: Basal together with pentagastrin-stimulated hCT measurement in case of basal hCT >10 pg/ml detect MTC in 0.20% of patients with nodular thyroid disease. Whether this high incidence of MTC has major implications or not has to be discussed, but should be considered as a useful and recommended tool for early detection of MTC and to save the patient’s life.
Introduction

Thyroid nodules are frequently observed in clinical practice with a prevalence of about 23% in Germany\(^1\). Calcitonin (CT) is a 32 aminoacid polypeptide secreted mainly by the parafollicular C cells of the thyroid and medullary thyroid carcinomas (MTC) are derived from the same cells and uniformly express CT\(^2\). MTC reportedly accounts for approximately 5% of all thyroid carcinoma and is sporadic in 50-78% of cases\(^3-7\). MTC is characterized by early micrometastasis and a lack of curative non-surgical treatment, so that early diagnosis is desirable. By the time patients with MTC present with clinical disease, the condition is usually metastastic and cannot be cured by surgery.

An elevated hCT value in patients with thyroid nodules after exclusion of potential condition (impaired renal function, bacterial infection, alcohol- and drug-abuse, pseudohypoparathyroidism, or proton pump inhibitor therapy) needs re-testing after an iv pentagastrin. HCT, stored in dense-cored secretory granules, can be released into the bloodstream with the synthetic analog gastrin pentapeptid (pentagastrin)\(^8\). Pentagastrin binds to the extracellular domain of the transmembrane cholecystokinin (CCK)-B/gastrin receptor and stimulates hCT secretion\(^9\). Therefore, hCT measurements after pentagastrin testing are used for biochemical diagnosis of primary and/or occult MTC.

In spite the fact that many European consensus groups and societies recommended serum calcitonin (hCT) measurement as screening parameter detecting medullary thyroid cancer the majority of physicians do not routinely use this testing\(^10, 11\). In the past years, several studies revealed different upper limits of basal hCT values to detect MTC and to reduce false-positive cases\(^12-15\). The present study sheds further light in the hCT measurement and its testing after pentagastrin stimulation in patients with thyroid nodule disease.
Patients and Methods

1007 patients (667 females, 440 males, mean age of 55±14 years, median 56 years) with nodular thyroid disease found by sonography living in central Germany, an area with endemic goiter due to previous iodine deficiency, were included in the study between June 2005 and September 2009. In one single center (Div. of Endocrinology, Technology Center Bochum, Germany) patients with known or unknown thyroid disease underwent ultrasound. In case of thyroid nodules, measurement of hCT was performed and was the first hCT determination in every patient. Individuals referred with known elevation of hCT, Graves´ disease or autoimmune thyroid disease were not considered or included to be part of this investigation.

All patients did not have impaired renal function, bacterial infection, alcohol- and drug-abuse, pseudohypoparathyroidism, or proton pump inhibitor therapy. hCT was measured in the Institution of Endocrinology and Laboratory in the Technology Center of Bochum, Germany, with the solid-phase, enzyme-labeled, two-site chemiluminescent assay with the immulite 2000 (Siemens immulite 2000, Munich, Germany). When basal calcitonin values were more than or equal to 10 and less than 100 pg/ml, testing was repeated 2 and 5 min after pentagastrin stimulation (with an iv bolus injection of 0.5 µg pentagastrin (Peptavlon®; Laboratoires SERB, Paris, France) per kilogram body weight) at the same institution. Basal or stimulated levels more than 100 pg/ml were indicated for surgery.

In case of patients with elevated pentagastrin-stimulated hCT-levels >100 pg/dl or basal hCT >100 pg/dl total thyroidectomy was performed. Moreover, both recurrent nerves were dissected carefully and a systemic microdissection of the central lymph node compartments along both nerves from the upper thoracic outlet up to the larynx was done. Germ-line mutations of the RET proto-oncogene were investigated in all patients with MTC.

All patients have given informed consent to the diagnostic and therapeutic procedures. For retrospective analysis of existing data sets from routine patients care, no institutional review board approval is required under German law and applicable institutional regulations.
Results

hCT levels >10 pg/ml were increased in 17 patients (1.7%) (figure 1). One patient had a basal hCT level of 4400 pg/ml with a histological confirmation of a MTC (table 1). In this patient, pentagastrin test was not performed. 16 patients with basal hCT between 10-100 pg/ml underwent pentagastrin-stimulated hCT measurement (figure 2). The mean increase of hCT after pentagastrin stimulation (table 1) was 4.6-fold (range 2.6 to 7.9) in patients 1-12, 8.8-fold (8.5 to 8.8) in patients 13-15 (CCH) and 25-fold in patient 16 (MTC).

All patients with basal or stimulated hCT >100 pg/ml could be observed for follow-up and underwent total thyroidectomy as well as the systemic microdissection of the defined regions. No permanent paralysis of the recurrent nerves was seen. One patient had permanent hypoparathyroidism. 5/17 had basal/stimulated hCT >100 pg/ml. 2/17 patients had MTC and 3/17 patients C cell hyperplasia. In sum, 2 patients (0.20%) had histological verified MTC. No mutation of the RET-proto-oncogene could be detected, so that the 2 MTCs were classified as sporadic.16,17

The age of the males with MTC was 76 and of the female 66 years. Basal hCT levels of the patients were 58 and 4400 pg/ml. Postoperative, basal hCTs of the patients with CCH were <2 pg/ml and of patients with MTC 2 and 11 pg/ml (table 1). In this last patient (#16), stimulated hCT after pentagastrin testing increased to 200 pg/ml. In patient #17 with basal preoperative hCT of 4400 pg/ml, postoperative pentagastrin stimulated hCT was 21 pg/ml. Tumor size of patient #16 was 3 mm and of patient #17 with MTC 20 mm. The 5 patients with CCH and MTC were diagnosed by histology from two different pathologists and were verified of a third pathologist, who has seen tissues from all 5 patients.

To date, positron emission tomography (PET) investigation using fluorodeoxyglucose (FDG) as the most sensitive and specific single modality, could not detect metastases in this patient.18 Patients with stimulated hCT <100 pg/ml did not underwent surgery of the thyroid and were under follows up with re-testing.
Discussion

The present study has shown, that hCT screening disease revealed one case of MTC among 500 patients with thyroid nodule disease. The present MTC-prevalence of 0.20% in patients with thyroid nodule disease is lower but also similar to some previous studies (0.33-0.40%), in which patients with known hypercalcitonemia were excluded\textsuperscript{13, 19, 20}. A higher prevalence rate have been found in several previous studies, which may be due to patient selection and/or to different normal range values employed determined by RIA or IRMA\textsuperscript{14, 21-23}. The group of Vierhapper has shown that before hCT screening was instituted, the diagnosis of MTC was made in 1 out of 900 patients with thyroid nodule disease referred to their clinic\textsuperscript{13}. The determination of basal hCT was responsible for the 3-fold increase to diagnose MTC. Moreover, the increase of quality of ultrasonography is a pivotal importance for the outcome of a hCT screening program as long as hCT is only determined in patients with thyroid nodule disease.

The cut-off level of hCT to initiate pentagastrin testing remains controversial, because basal hCT values between 10 and 100 represent a gray-zone where true and false positive cases overlap\textsuperscript{12, 22, 24-26}. We have performed pentagastrin testing in all patients with hCT >10 pg/ml without loss of follow-up of any patients. 4 of 16 patients had hCT >100 pg/ml after pentagastrin stimulation and underwent thyroidectomy with the result of 1 MTC and 2 C cell hyperplasia. In a recent study of Rink et al., the increase of the upper limit for basal hCT to 15 pg/ml, instead of 10 pg/ml, significantly reduced the number of false-positive cases\textsuperscript{12}. None of these patients with basal hCT <10 pg/dl had MTC. In our study, all patients with basal hCT >15 pg/ml and pentagastrin stimulated hCT >100 pg/ml had confirmed CCH or MTC. On the other hand, in another study from Vierhapper et al., basal hCT <15 pg/dl was stimulated by pentagastrin >100 pg/dl with histological confirmation of MTC in one patient\textsuperscript{13}. Moreover, in the study from Rink et al. one patient had basal hCT >15 pg/ml and pentagastrin stimulated hCT of 81 pg/dl with histological confirmed MTC, so that the cutoff levels of pentagastrin stimulated hCT >100 pg/dl did not have 100 % sensitivity\textsuperscript{12}. Moreover, stimulated hCT >100 pg/ml cannot differentiate between CCH and MTC, considering the histological results with CCH of 3 patients of our patients with stimulated hCT between 176 and 322 pg/ml.
A recent study from Doyle et al. investigated stimulated hCT after high-dose calcium in comparison to pentagastrin. Calcium seemed to be a more potent and better-tolerated hCT stimulator than pentagastrin, and could be considered as a new and valid alternative in future\textsuperscript{27}.

Moreover, men and women differ in thyroid C cell mass and calcitonin secretion. To increase the positive predictive value, it is to discuss and to consider that there exists a gender-specific threshold predicting sporadic occult MTC\textsuperscript{28}.

Nevertheless, the risk of MTC is obviously very low in this range of 10-15 pg/dl of basal hCT and <100 pg/dl after pentagastrin stimulation. This is in accordance with the upper limit of the normal range of 100 pg/dl, as published in the German consensus recommendation of 2004\textsuperscript{10}. The recommended yearly follow-up investigations in patients with pentagastrin stimulation of hCT between 50 and 100 pg/dl can shed new light to this issue. To lower this threshold for surgery may refer to many patients to unnecessary operations. Thus the definition of 100 pg/dl as therapeutic threshold reflects a clinical compromise, balancing over- and undertreatment in these patients.

C cell hyperplasia (CCH) was seen in three patients with (0.3%) similar to previous observations (0.5\%)\textsuperscript{13}. CCH is characterized by an increased quantity of C cells within the thyroid, commonly defined as >50 C cells per low power field in histological examination and has been considered as carcinoma in situ of the thyroid parafollicular cells in hereditary MTC\textsuperscript{29, 30}. In contrast, the clinical relevance of sporadic CCH outside hereditary MTC remains unclear, and the hypothetical role of CCH as a risk factor for sporadic C cell malignancy is not supported by available evidence\textsuperscript{11, 31}.

Determination of serum hCT is more sensitive than fine-needle aspiration MTC detection\textsuperscript{14, 32}. False negative reports of fine-needle aspiration may occur in case of thyroid malignancy, both in large nodules and microcarcinomas, tumors <10 mm in diameters\textsuperscript{33}. Nevertheless, fine-needle aspiration
should be recommended considering the fact that one of the two MTCs in our study was 3 mm and could be detected positively by cytology.

The age of our patients with MTC (66 and 76 years) is higher than the known mean age of sporadic MTC (46 years) which may be due to the mean age of the patient in our institution and may not reflect a more benign form of MTC in these patients discovered by screening\textsuperscript{34}.

Whether tumors with small size could stay potentially in their tumor stage for a very long time or may develop metastases cannot be answered, but has to be considered. It has been shown, that preoperative hCT levels correlated with the postoperative tumor size in patients with MTC, and its relationship was straighter in familiar than in sporadic forms\textsuperscript{23, 35}. Both of our two patients with MTC had the sporadic form, which could be an explanation of the missing correlation of hCT levels and tumor size. One patient with 20 mm tumor size without metastases could be cured by surgery, although the preoperative hCT was very high (4.440 pg/ml) with suggested metastatic disease. But postoperative basal and pentagastrin stimulated hCT <10 pg/ml documented the cure of this patient. The other patient with a 3 mm tumor size had already lymph nodes metastases and could not be cured by surgery (preoperative stimulated hCT >1.471 pg/ml), so that initial tumor size cannot predict metastases or not.

In conclusion, primarily basal hCT measurement is recommended in patients with thyroid nodule disease. It has been shown, that one case of MTC among 500 patients with thyroid nodule disease could be detected in an early tumor stage. Whether this high incidence of MTC has major implications or not has to be discussed, but should be considered as a useful and recommended tool for early detection of MTC and to save the patient’s life. To screen with the cost-effective hCT measurement can help the overwhelming majority of these patients with cure by surgery.
Disclosure Statement

The authors declare that no competing financial interests exist.

This research did not receive any specific grant from any funding agency in the public, commercial or not-for-profit sector.
References


Fig. 1: Serum calcitonin levels under basal conditions in 1007 patients with nodular thyroid disease

Fig. 2: Calcitonin levels with pentagastrin testing in 16 patients with initial basal calcitonin values >10 pg/ml and <100 pg/ml.
Fig. 1
Fig. 2
Table 1  Patients characteristic with elevated hCT (>10 pg/dl)

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**Age [years]**
M: male; F: female
hCTb: basal hCT [pg/ml]
hCTs: maximal pentagastrin-stimulated hCT [pg/ml]
Sono: size of the dominant nodule by sonography [mm]
FNAB: fine needle aspiration
CCH: C cell hyperplasia
hCTpost: postoperative hCT [pg/ml]