Calcitonin Measurements for Early Detection of Medullary Thyroid Carcinoma or its Premalignant Conditions in Hashimoto’s Thyroiditis

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Abstract. The measurement of basal serum calcitonin (CT) in patients with evidence of Hashimoto’s thyroiditis (HT) has been proposed in a recent study demonstrating an increased prevalence of elevated basal and stimulated CT. The aim of this study was to evaluate the frequency and relevance of elevated CT levels in HT. The basal sera CT were measured in 568 consecutive HT patients using a chemiluminescent immuno-assay. Whenever the serum CT was >10 pg/ml, a pentagastrin (PG) stimulation test was performed. Two patients with abnormal/pathological PG tests were identified. Total thyroidectomy and lymph node dissection revealed for the first patient medullary thyroid carcinoma (MTC) and for the second patient C cell hyperplasia (CCH), together with papillary thyroid carcinoma. Our data showed a low prevalence of MTC and its premalignant condition CCH in HT patients; nevertheless, the patient with MTC presented lymph node metastasis. The fact that both cases presented without evidence of nodular thyroid disease highlights the persistent diagnostic dilemma of CT screening programs.

Serum calcitonin (CT) is a sensitive and accurate marker of medullary thyroid carcinoma (MTC), and a significant increase in CT after administration of pentagastrin (PG) is a specific feature of MTC (1-3). Patients with nodular thyroid disease (4, 5), showing basal and stimulated plasma CT levels of more than 100 pg/ml, should be considered for operation (6) because they most probably suffer either from MTC or C cell hyperplasia (CCH), a potentially precancerous lesion (7). This strategy increases the probability of early diagnosis of MTC, thus providing the chance of curative surgery (6). Recent data published by our group (8) suggest that basal CT measurements could be of use in the detection/screening of MTC, not only in subjects with nodular thyroid disorders, but also in patients with immunological evidence of Hashimoto’s thyroiditis (HT).

The aim of our trial was to evaluate the relevance of routine CT measurements for detection of MTC or its premalignant associated conditions (micro-MTC and neoplastic C cell hyperplasia) in HT patients.

Patients and Methods

Patients with Hashimoto’s thyroiditis (HT). Five hundred and sixty-eight consecutive patients with HT (68 males and 500 females), aged from 18 to 88 years (mean age 55 years), from our out-patient department, were included in the present study (Table I). All patients underwent thyroid palpation, sonography and laboratory analyses of thyreotropin, free thyroxine, triiodothyronine and autoantibodies to thyreoglobulin (TG) and antithyroid peroxidase (TPO), measured with commercial kits by an IMMULITE® 2000 (EURO/DPC, Gwynedd, UK). All patients enrolled in this study fulfilled the following criteria: documented history of positivity for TPOAb, negativity for anti-TSH receptor antibodies and thyroid ultrasound imaging suggestive of a chronic thyroiditis.

The serum CT was determined in each patient with a commercial assay by a Nichols Advantage Chemiluminescence System (Nichols Institute Diagnostics, San Juan Capistrano, CA, USA). The analytical sensitivity was 1 pg/ml. The coefficients of variation (CV) were 3.5% / 6.2% (intra-assay) and 5.1% / 8.7% (between day variation) at average CT levels of 553 pg/ml and 10 pg/ml, respectively. Elevated CT was confirmed by an additional dilution test. In rare cases of nonconformity, the result of the
Sonography. Thyroid sonography was performed in all patients, by one experienced physician, with a Siemens Sonoline Prima diagnostic ultrasound system (Siemens, Erlangen, Germany) or LOGIC 400 pro series (GE, USA), using a linear 7.5MHz transducer.

Surgery. By definition (6), patients with abnormal and pathological PG tests were candidates for surgery. Patients with abnormal PG test results were treated by total thyroidectomy and lymph node dissection along both recurrent nerves (6). In patients with abnormal PG testing and intraoperatively verified MTC, as well as in patients with pathological testing on both sides, a lateral lymph node dissection from the skull to the thoracic outlet was performed.

Histology and molecular genetic analysis. Entirely blocked and hematoxylin and cosin-stained thyroidectomy specimens were investigated by immunohistochemistry using the avidin-biotin-peroxidase method. The CT antibody (Chemicon, Temecula, CA, USA) was diluted 1:600. CCH was defined according to the criteria of Rosai et al. (10), if more than 50 C cells in a single low-power field (x100 magnification) in both thyroid lobes were detected.

The search for germ-line mutations of the RET proto-oncogene should exclude hereditary types of C-cell pathology in patients undergoing surgery by analyzing its exons 10, 11, 13, 14, 15 and 16. DNA was extracted from properly masked peripheral blood (DNAzol, Vienna Lab, Vienna, Austria). Polymerase chain reaction amplification and DNA sequencing were performed, as described previously (11).

Results

CT levels in HT patients. As reported in Table I, 463 patients (81.5%) were free from nodules verified by sonography, while the remaining 105 patients (18.5%) showed nodular structures. Fourteen out of 568 patients (2.5%) presented with elevated basal serum CT levels, 9 (64.3%) of them without, and the remaining 5 (35.7%) with, nodular thyroid structures (Table II). All nodules in HT patients with elevated serum CT levels were hypoechoic/circumscribable and their maximum diameter was 13±5 mm (range: 4.5-17 mm). The ratio between males and females was 2:3 and 3:6 for patients with and without nodular HT, respectively.

Neither age nor thyroid function were significantly different in both groups (Table II), but the thyroid volume was significantly higher in nodular than in non-nodular HT patients (16±6 versus 8±4 ml; Table II). Basal CT and PG-stimulated CT did not differ significantly between both groups (Figure 1).

Two out of 14 HT patients (0.35% of all HT patients) with elevated basal CT levels revealed an increase of CT >100 pg/ml after PG stimulation. An abnormal stimulated CT (125 pg/ml, 2 min after PG) was found in a 37-year-old male patient. The response of 1799 pg/ml CT 2 min after PG of a 57-year-old female was rated pathologically. Both patients were free of nodules, verified by sonography, and underwent surgery.

The male patient suffered from a papillary thyroid carcinoma (PTC) (pT1b, N1a: 5/45, UICC 1997, largest cancer focus: 6 mm) together with a diffuse CCH (215 C cells/ single low-power field/x100 magnification) in both thyroid lobes were detected.

Discussion

A diagnosis of MTC in an early and, therefore, potentially surgically curable stage of the disease is essential to improve the prognosis (4, 5). Beside MTC, CT is also overexpressed by CCH, a potential precancerous condition even in the...
absence of germ-line mutations in the RET proto-oncogene (7, 12). Thus, CT screening in nodular thyroid disease has become a common (and established) approach. It is unclear yet, whether HT patients – with or without evidence of nodules – are candidates for CT screening (8). An association between CCH and HT has been described (13-16). Barbot et al. reported increased serum CT levels and extensive CCH in histology after thyroidectomy in 3 out of 24 patients with HT (16). In extended studies, Guyetant et al. (13) reported the occurrence of CCH in 20% of HT patients. The pathophysiological link between CCH and HT may involve an immunopathological mechanism, or an effect of the inflammatory mediators and cytokines secreted by infiltrating lymphocytes in the thyroid parenchyma. The role of a C-cell growth factor, whose gene is overexpressed in hyperplastic C-cells adjacent to follicular tumors, is

<table>
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Values are given as absolute numbers (%) if not otherwise specified.
The significance of differences between the groups was determined by a two-tailed t-test with a rejection level of 0.05.

Figure 1. Basal and stimulated CT (pg/ml) in the fourteen patients with elevated CT levels.
hypothesized (13). Interestingly, basal CT levels were related to stimulated CT levels in non-nodular but not in nodular thyroid disease. The PG-stimulated increase in CT was higher in non-nodular thyroid disease. Perry et al. defined the differences between physiological and neoplastic CCH (17). One might hypothesize that the increase in CT reflects a cause-effect relationship in HT, a physiological response of all C-cells in the thyroid gland in non-nodular disease. Yet no biochemical markers exist for the distinction between physiological and neoplastic CCH.

The prevalence of elevated basal CT levels was 2.5% (14/568) in this study. Histologically verified CCH or MTC were detected in 0.18% (1/568) each, which was lower than in previously published trials, ranging from 0.24% to 1.37% (8, 18). In a previous study (8), the prevalence of elevated basal CT levels in patients with nodular and non-nodular thyroid disease was 6.8%, of CCH 0.72% and of histologically verified MTC 0.24%. The selection of study groups, as well as the low prevalence of the disease, may explain these discrepancies. In this study, 2 HT patients, 1 with abnormal, the other with pathological PG testing, were identified. Neither of them had evidence of nodules by palpation and sonography and would have been missed by these clinical criteria alone. One of them revealed CCH and a co-existing multifocal micro-PTC (with 5 lymph nodes metastasis). The final diagnosis of the other 1 was MTC with 2 lymph nodes metastasis.

Identification of subjects at risk for MTC relies on an appropriate cut-off value for basal and stimulated CT. To stimulate CT secretion, pentagastrin is routinely used and criteria for test interpretation have been established, which have been clinically validated (6). However, less is known about the physiological variation in CT levels. They might be related to the subject’s nutritional status (fasting/non-fasting), or influenced by blood sample collection. In our institution, as in many others, the basal CT was measured first during the thyroid work-up in a non-fasting state with the patient seated. This is in contrast to the standardized conditions during PG stimulation testing with the fasting patient lying down. These differences might account for the variation in basal CT levels which were observed. Five out of 14 patients had basal CT values ≤10 pg/ml at the time of PG stimulation.

The co-existence of CCH and PTC seems a rare condition, which has been mentioned in only a few reports (19-21). It was speculated that CCH is causally related to pathological disorders affecting follicular cells (19), or that a common tumorigenic stimulus triggers the neoplastic transformation of both parafollicular C cells and follicular epithelial cells (20), or that the pathogenesis of PTC is linked to exon 11, 13, 14 and 15- RET mutations that affect the intracellular domain of the encoded protein (21).

The costs for CT screening in this HT study were about 29,000€ per MTC patient identified, but were reduced if CCH was included. The 10-year survival rate of sporadic MTC is believed to be only about 60 %, due to the advanced stage of the disease at diagnosis in the majority of patients. Although the cost for the detection of MTC seems very high, CT screening was of tremendous benefit. At the time of diagnosing HT in our female patient, she was not a candidate for surgery, but the elevated basal and stimulated CT levels, indicated preoperatively a suspected malignancy. An enforced screening for MTC, or its premalignant condition CCH, may increase the life expectancy of MTC, in HT patients as well.

Meticulous neck dissection, performed routinely in MTC but not in benign conditions, documented 2 metastasis out of 94 resected lymph nodes. One year later, basal and stimulated CT levels were undetectable, thus documenting a biochemical cure. Nevertheless, the patient is at risk of developing recurrence (22).

**Conclusion**

Our data showed a low prevalence of MTC, and CCH as its premalignant lesion, in HT patients. The overall prevalence of MTC and CCH in HT patients was 0.35% and, therefore, even lower than in patients with nodular disease. The fact that 2 patients presented without evidence of nodular thyroid disease – 1 of them with documented MTC and lymph node metastasis – highlights the persistent discussion about the diagnostic value of CT screening in all thyroid abnormalities.

**References**


Received July 20, 2005
Revised September 21, 2005
Accepted September 27, 2005