Value of routine measurement of serum calcitonin concentrations in patients with nodular thyroid disease: A multicenter study

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ABSTRACT. ***Background:*** **The routine measurement of serum calcitonin (CT) has been proposed for patients with nodular thyroid disease (NTD), to detect unsuspected medullary thyroid carcinoma (MTC) before surgery.** ***Objective:*** **To assess the prevalence of hypercalcitoninemia and MTC in NTD patients; to compare the ability of CT measurement and fine needle aspiration cytology (FNAC) to predict MTC; to identify age groups of NTD patients who should be better candidates than others to undergo routine measurement of CT.** ***Patients and methods:*** **1425 consecutive patients, referred from April 1, 2003, through March 31, 2004, to four Italian endocrine centers due to NTD, were grouped depending on age, and underwent basal and, in some cases, pentagastrin (Pg)-stimulated CT measurement, FNAC and, when indicated, surgery. Serum CT concentrations were measured by an immunoluminometric assay (ILMA).** ***Results:*** **Hypercalcitoninemia was found in 23 out of 1425 patients. MTC was discovered in 9 patients, all >40 yr old and showing high CT levels. Sensitivity of basal and Pg-stimulated CT to predict MTC before surgery was 100% for both tests, whereas specificity was 95 and 93%, respectively. CT specificity reached 100% when a cut-off value of 20 pg/ml was taken. FNAC showed an overall 86% sensitivity. When >10 mm MTC nodules were considered, FNAC sensitivity approached 100%. On the contrary, a correct cytological diagnosis was obtained in only one out of five patients with <10 mm MTC nodules (microMTC); in one patient with histologically proved microMTC, FNAC even demonstrated a benign lesion. Hypercalcitoninemia or MTC were associated with chronic thyroiditis in 30 or 33% of cases, respectively. C-cell hyperplasia was found in 57% of hypercalcitoninemic patients without MTC.** ***Conclusions:*** **Basal CT measurement detects elevated CT values in 1.6% of NTD patients. Although CT is not a specific marker of MTC, its routine measurement represents a useful tool in the pre-operative evaluation of NTD patients, particularly those >40 yr old presenting with nodules <10 mm, even when FNAC does not show malignant features. To our knowledge, this is the first trial using ILMA to assess the ability of pre-operative CT measurement to predict MTC in a large series of NTD patients.**

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INTRODUCTION

Patients with nodular thyroid disease (NTD) are frequently observed in the clinical practice. In countries with iodine sufficiency, 4 to 7% of the adult population have a palpable thyroid nodule (1, 2). Prevalence of thyroid nodules in ultrasound or autopsy studies approaches 50% of subjects aged ≥60 yr (1, 3-5); however, <5% of nodules are malignant (6).

Key-words: Calcitonin, fine needle aspiration cytology, thyroid nodules, medullary thyroid carcinoma.

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Medullary thyroid carcinoma (MTC) is a malignant tumor of the thyroid gland that appears as nodule(s), and represents approximately 5% of all thyroid neoplasms (7). MTC arises from the neural crest-derived parafollicular C-cells. About 30-50% of patients with MTC have a familial form with a genetic mutation and the remainder has sporadic disease (8, 9). It has been demonstrated that distinct germline mutations in the *RET* proto-oncogene – which encodes a transmembrane receptor with cytoplasmic tyrosine kinase activity – are associated with the dominantly inherited cancer syndromes multiple endocrine neoplasia types 2A and 2B (MEN 2A and MEN 2B) and familial medullary thyroid carcinoma (FMTC), all sharing MTC as part of the disease phenotype (9).

MTC is more aggressive than papillary and follicular thyroid carcinomas; survival rate at 10 yr is 40-50%, strongly depending on the tumor stage at diagnosis (10). It has been clearly demonstrated that when the neoplasm is confined within the thyroid, and thyroidectomy with dissection of the central lymph node compartment is performed, the chance of definitive cure is high (11, 12). Hence, early detection of MTC is crucial to save the patient's life.

Actually, two diagnostic tools are available in the evaluation of patients with NTD, included those with MTC: fine needle aspiration cytology (FNAC) and measurement of basal human calcitonin (CT) concentrations. FNAC is able to distinguish benign from malignant nodules in most NTD patients; however, false negative reports may occur in case of thyroid malignancy, both in large nodules and microcarcinomas, tumors <10 mm in diameter (13, 14). Elevated CT levels identify almost 100% of MTC, even at the stage of microcarcinomas (15, 16). Based on this evidence, some authors have recently proposed routine measurement of serum CT for all patients with NTD. However, elevated CT levels are not observed only in MTC but also in benign diseases, mainly chronic thyroiditis (17-21). In contrast, other authors do not favor the routine measurement of CT in patients with NTD, because of the high prevalence of thyroid nodules in the general population, rarity of MTC, low specificity of basal CT values to predict MTC, and cost of screening (4, 22, 23). Because of these limitations, routine CT measurement has resulted in a lack of general acceptance in US (24).

The aim of this multicenter study was: 1) to assess the prevalence of hypercalcitoninemia and MTC in a large series of patients with NTD; 2) to compare sensitivity and specificity of routine measurement of CT and FNAC in the pre-operative diagnosis of unsuspected, sporadic MTC; 3) to identify age groups of patients who should be better candidates than others to undergo routine measurement of CT in the pre-operative evaluation of NTD.

PATIENTS AND METHODS

Study design

During the period April 1, 2003, through March 31, 2004, 3458 patients with suspected thyroid diseases were referred to the Division of Endocrinology, "A. Gemelli" Hospital - Catholic University of Rome, the Division of Endocrinology, University Hospital - University of Modena and Reggio Emilia, the Department of Internal Medicine, "Estense" Hospital - AUSL Modena, and the Department of Internal Medicine, "Ramazzini" Hospital - AUSL Modena.

Inclusion criteria were: 1) male and female patients aged ≥18 yr; 2) palpable nodule(s); 3) non-palpable nodules ≥10 mm in their greatest diameter; 4) non palpable nodule(s) <10 mm in their greatest diameter, showing ultrasound features suspicious for malignancy (microcalcifications, undefined margins, intra-nodular vascularization).

Exclusion criteria were: 1) patients <18 yr old; 2) non-palpable nodule(s) <10 mm in their largest diameter, without ultrasound features suspicious for malignancy; 3) patients previously evaluated for nodular goiter by FNAC and/or CT measurement; 4) patients reporting a family history of MEN (2 cases of MEN 2A) and those previously thyroidectomized for sporadic MTC (one case); 5) patients with hyper- and hypothyroidism without nodules; 6) patients in follow-up for thyroid diseases; 7) patients not confirmed as having thyroid diseases.

Patients

In total, 1474 patients fulfilled the criteria of the study design but 49 dropped out of the study, either because they refused FNAC and/or surgery (no.=32) or because they were lost following the first visit (no.=17). Of the remaining 1425 patients, 432 (30%) were recruited by "A. Gemelli" Hospital (Rome), 612 (43%) by University Hospital and "Estense" Hospital of Modena (considered together, because they shared the same staff of pathologists), and 381 (27%) by "Ramazzini" Hospital of AUSL Modena. When matched for gender, age, nodule size, CT and TSH concentrations, no significant differences (p=ns) were found among patients recruited by different institutions.

All patients satisfying inclusion criteria underwent the following procedures: a) blood sample for measurement of serum TSH and CT concentrations; b) ultrasound-guided FNAC.

When TSH value was abnormal, serum free-T_4 (FT_4) and free-T_3 (FT_3) concentrations were also measured. Furthermore, patients with suppressed serum TSH concentrations underwent ^{99m}Technetium-thyroid scan, to detect autonomously functioning nodules.

Whenever basal serum CT concentrations exceeded 5 pg/ml, both the measurement of thyroid antibodies – ie, antibodies against thyroglobulin (TG-Ab) and thyroperoxidase (TPO-Ab) – and a pentagastrin (Pg) stimulation test were performed. Because in the experience of our centers a basal CT level >100 pg/ml always predicts MTC, the Pg-stimulation test was not performed when basal CT values exceeded 100 pg/ml. During Pg stimulation test, some patients experienced nausea, dizziness and tachycardia. None of the patients had clinical conditions contraindicating the test.

Finally, patients were arbitrarily divided into four groups, based on age at first observation: 18-39 yr (group 1); 40-49 yr (group 2); 50-64 yr (group 3); ≥65 yr (group 4).

Surgery was always recommended if one of the following criteria was met: a) FNAC diagnostic or suspicious for malignancy (indeterminate picture included); b) repeatedly (>2) non-diagnostic FNAC; c) basal serum CT concentrations >5<100 pg/ml and Pg-stimulated CT levels ≥100 pg/ml; d) basal serum CT concentrations ≥100 pg/ml; e) complaints of compressive symptoms (dysphagia, dyspnea). All patients gave informed consent to the diagnostic and therapeutic procedures.

Methods

CT assay

Basal and Pg-stimulated serum CT concentration was measured in the laboratories of the Institutions involved in the study, using the same two-site chemiluminescence immunoassay (immunoluminometric assay, ILMA, Nichols Institute Diagnostics, San Juan Capristano, CA 92675, USA). The ILMA utilizes two mouse monoclonal antibodies against CT. One of the mouse monoclonal antibodies is labeled with acridinium ester for detection, while a second mouse monoclonal antibody is coupled to biotin. CT is "sandwiched" between these antibodies. The ILMA is specific for the mature CT monomer, and has a sensitivity of 1 pg/ml. The intra-assay variation is 6.2% for 12.1 pg/ml, 3.5% for 95.5 pg/ml, and 3.5% for 544 pg/ml .The inter-assay variation is 8.8% for 10.5 pg/ml, 7.3% for 85.7 pg/ml, and 4.5% for 527 pg/ml. The assay does not show cross-reaction with PTH, insulin, PRL, GH, TSH, ACTH, C-peptide, C-procalcitonin, chicken CT, porcine CT and salmon CT, whereas a 0.0002% cross-reactivity with calcitonin gene-related peptide (CGRP) has been reported by the manufacturer. Bilirubin exceeding 10 mg/dl interferes in the measurement of CT; no patient of our series had high bilirubin levels. The upper limit of normal range in our laboratories was established to be equal to 5 pg/ml.

Pg stimulation CT test

For the Pg stimulation test, blood for the measurement of CT was collected before, and 2, 5, and 10 min after the iv injection of Pg (Cambridge laboratories, Wallsend, Tyne and Wear, NE28 9NX), 0.5 mcg/kg of body weight. The response was expressed as the maximum value (peak) of CT measured either 2 or 5 min following Pg injection; in our laboratories, a value ≥100 pg/ml is considered abnormal.

Fine needle aspiration biopsy

Ultrasound-guided fine-needle aspiration biopsy was performed with 23-27 gauge needles on nodular areas. Multiple direct smears were prepared, and May-Grumwald-Giemsa and Papanicolau stainings were employed.

Surgery

Patients with a benign FNAC and compressive symptoms underwent lobectomy or near-total thyroidectomy, depending upon the presence of monolobar or bilobar nodular involvement, respectively. In case of suspicious or repeatedly non-diagnostic FNAC, frozen sections of the nodule(s) were obtained for intraoperative histological examination, to decide on the definitive surgical extension. In all patients undergoing surgery following a cytological diagnosis of malignancy other than MTC, total thyroidectomy was the procedure of choice completed by a lymph-adenectomy in case of lymph node involvement. In patients with basal CT values ≥100 pg/ml, or basal CT values >5<100 pg/ml and Pg-stimulated CT levels ≥100 pg/ml, and/or a cytological diagnosis consistent with MTC, a total thyroidectomy was performed. In these cases a systematic microdissection of both central neck and bilateral neck compartments was made. Contralateral lymph node dissection was omitted in patients with a unilateral thyroid tumor and no ipsilateral and central lymph node involvement.

Histological and immunohistochemical examination of surgical specimens

The surgical specimens were examined using a standard histological technique. Microscopic slides were obtained from formalin-fixed and paraffin-embedded tissue, and stained with H-E. In all patients with high basal CT values, the slides were also stained with Congo red, and immunohistochemical studies were performed, by an avidin-biotin-peroxidase method and a panel of commercially available antibodies directed against CT (Euro Diagnostica, Milan, Italy), to identify possible cases of MTC, and/or C-cell hyperplasia (CCH). Furthermore, the slides of 7 patients with normal basal CT concentration and chronic thyroiditis were also stained by CT antibodies to detect areas of CCH. All pathologists involved in the study defined the presence of CCH when more than 50 C cells were found in a single low power field (x100 magnification) in both thyroid lobes (25). Cancers were staged according to the 6th edition of Cancer staging manual (26).

Molecular genetic analysis

Investigation for germline mutations of the *RET* proto-oncogene was performed in patients with histologically proved MTC, by analyzing exons 10, 11, 13, 14, and 16 of this gene. Polymerase chain reactions (PCR) were carried out under standard conditions as previously described (27).

Screening for hyperparathyroidism and pheocromocytoma

In all patients undergoing surgery because of elevated basal CT values, intact PTH, serum calcium levels, and urinary metanephrine and catecholamine levels by high performance liquid chromatography (HPLC), were measured to exclude hyperparathyroidism and pheocromocytoma, respectively.

Statistical analysis

Results are reported as mean±SD. The different groups of patients were compared using analysis of variance (ANOVA) followed by paired and unpaired Student's t-test. Ordinal variables were analyzed using the χ^2 (chi-square) test. Statistical significance was taken as $p<0.05$. All statistics were conducted using the commercially available software program SPSS for Windows, release 10.0 (SPSS Inc., Chicago, IL, USA). To determine sensibility, specificity, and accuracy of FNAC in the diagnosis of MTC, cases diagnosed with, or suspicious for tumors other than MTC and those classified as non-diagnostic, were excluded from calculation because these results are not sufficient to program the appropriate thyroidectomy required for MTC; cases with definitive diagnosis of, or suspicious for MTC were considered as "positive".

RESULTS

Whole population

Data related to the four groups of patients are reported in Table 1.

Of 1425 patients who fulfilled the criteria of study protocol, there were 281 males (20%) and 1144 females (80%), with a female to male ratio of 4:1, ranging in age from 18 to 91 yr (mean: 49.6±6.8 yr; median: 51 yr). One thousand three hundred and seventy-nine patients (96.1%) were euthyroid, 32 (2.2%) hypothyroid, and 24 (1.7%) thyrotoxic (1 patient had sub-acute de Quervain's thyroiditis, 2 patients Graves' disease, and 21 patients toxic nodular goiter). Ultrasound detected multiple nodules in 1024 patients (72%) and a solitary nodule in 401 patients (28%). The mean diameter of dominant nodule was 21.8±4 mm (range:

Table 1 - *Results in 1425 patients subdivided into 4 groups according to age.*

	Patients (no.)	Age (yr)	Calcitonin (pg/ml) with (and without) MTC	Nodule size (mm)
Group 1 (18-39 yr)	356	31±3.1[a]	2±0.7[+] (2±0.7*)	19.6±6.2*
Group 2 (40-49 yr)	516	44.1±3[b]	2.7±0.9** (2.6±0.8*)	23.8±7.7*
Group 3 (50-64 yr)	368	58.2±2.9[c]	7.6±3.8[#] (3.1±1.1*)	21.9±5.4*
Group 4 (≥65 yr)	185	72±2.3[d]	12.6±4.6[§] (3.6±1.5*)	22±5.1*
Total	1425	49.6±6.8	6.2±5.6 (2.8±0.9)	21.8±4

Data are expressed as mean±SD. *p=ns *vs* other groups. [a]p=0.003 *vs* group 2, <0.0001 *vs* groups 3 and 4. [b]p=0.003 *vs* group 1, =0.001 *vs* group 3, <0.0001 *vs* group 4. [c]p<0.0001 *vs* groups 1, =0.001 *vs* group 3, =0.002 *vs* group 4. [d]p=0.002 *vs* group 3, <0.0001 *vs* groups 1 and 2. [#]p=ns *vs* groups 1, 2, 4. [+]p=ns *vs* groups 2 and 3, =0.02 *vs* group 4. **p=ns *vs* group 1 and 3, =0.002 *vs* group 4. [§]p=0.002 *vs* group 2, =0.02 *vs* group 1, =ns *vs* group 3. The mean of calcitonin values was calculated both including and excluding patients with medullary thyroid carcinoma (MTC).

7-60 mm). Basal serum CT concentration was 6.2±5.6 pg/ml (median: 2.7 pg/ml). Serum CT concentration was normal in 1402 (98.4%), elevated (>5 pg/ml) in 23 patients (1.6%); it significantly increased with age, ranging from 2±0.7 pg/ml (group 1) to 12.6±4.6 pg/ml (group 4) (p=0.002). Excluding patients with histologically proven MTC, basal serum CT concentration was 2.8±0.9 pg/ml (median 2.8 pg/ml) and did not show any significant correlation with age. The results of cytological examination are reported in Figure 1. A repeatedly non diagnostic cytological picture was found in 60 (4.9%) patients; a benign nodule was diagnosed in 1276 (89.5%) subjects; FNAC was suspicious for malignancy (37 follicular neoplasm, 25 papillary thyroid carcinoma, 1 medullary thyroid carcinoma, 1 anaplastic thyroid carcinoma) in 64 (4.5%) patients, whereas it was diagnostic for malignancy (21 papillary thyroid carcinoma, 3 medullary thyroid carcinoma, 1 anaplastic thyroid carcinoma) in 25 (1.8%) patients. Of 1425 patients, 315 (22%) were submitted to surgery, 23 patients with elevated and 292 with normal basal serum CT concentrations.

Characteristics of 292 patients submitted to surgery with normal basal CT values

The age of the patients who underwent thyroidectomy was 50.5±5.6 yr (range 18-84; median: 49.5 years; p=ns *vs* whole population). Basal serum CT concentration was 2.3±0.9 pg/ml (median: 2.2 pg/ml; p=ns *vs* whole population). This value did not differ among the 4 age groups (2.1±0.4 pg/ml in group 1; 1.8±1 pg/ml in group 2; 3.1±0.6 pg/ml in group 3; 2.4±0.5 pg/ml in group 4). The diameter of dominant nodule was 22.5±4.9 mm (range 12-65; p=ns *vs* whole population), and did not significantly differ among the four groups. The results of FNAC are reported in Figure 1. A repeatedly non diagnostic cytological picture was found in 16 (5.5%) patients; a benign nodule was diagnosed in 201 (68.8%) subjects; FNAC was suspicious for malignancy (32 follicular neoplasm, 22 papillary thyroid carcinoma) in 54 (18.5%) patients, whereas it was diagnostic for malignancy [20 papillary thyroid carcinoma, 1 anaplastic thyroid carcinoma] in 21 (7.2%) patients. The results of histological examination are reported in Figure 2. Histology disclosed a benign disease in 260 patients (89%) and a thyroid carcinoma in 32 patients (11%). Of the patients with benign thyroid diseases, a nodular goiter was diagnosed in 245 cases (84%), follicular adenoma in 11 cases (4%) and Hurthle cell adenoma in 4 cases (1%). Of the patients with malignancy, 26 (9%) had papillary thyroid carcinoma (PTC), 4 (1.4%) follicular thyroid carcinoma, 1 (0.5%) Hurthle cell carcinoma, and 1 (0.5%) thyroid metastasis of urothelial sarcomatoid carcinoma.

Patients with elevated basal serum CT concentrations submitted to surgery

Data from each patient are reported in Table 2 and Table 3.

Of the 23 patients with elevated basal serum CT concentration, 15 (65%) were female and 8 (35%) male (female to male ratio of 2:1), aged 52.6±2.3 yr (range: 28-74; median: 49 yr; p=ns *vs* patients with normal CT values and whole population). One subject (4%) belonged to group 1, 11 (48%) to group 2, 7 (30%) to group 3, and 4 (18%) to group 4. Basal serum CT concentration was 206±90.8 pg/ml (range: 5.6-1500 pg/ml; median: 16 pg/ml; p<0.0001 *vs* all recruited patients and *vs* patients with normal basal CT values). Nineteen of the 23 subjects were submitted to the Pg-stimulation test, which resulted abnormal

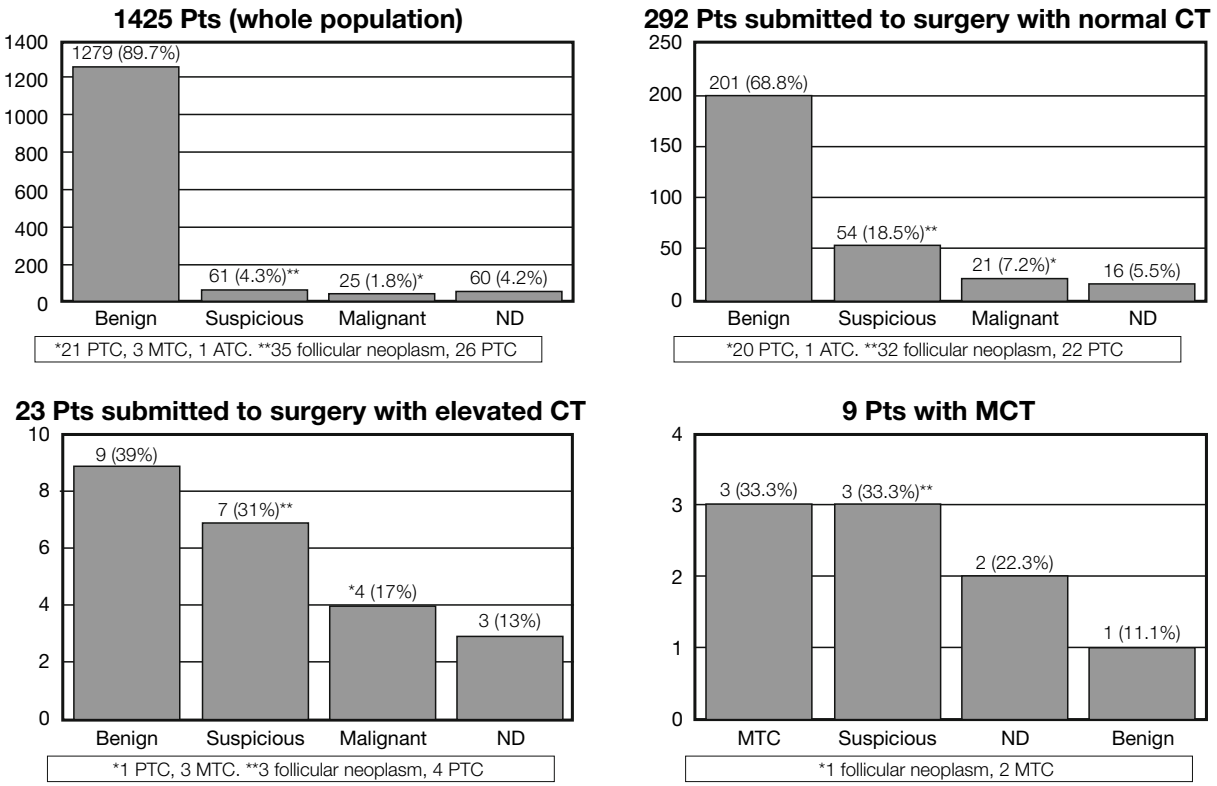

Fig. 1 - *Cytology reports in patients with nodular thyroid disease. ATC: anaplastic thyroid carcinoma; CT: calcitonin; FN: follicular neoplasm; MTC: medullary thyroid carcinoma; PTC: papillary thyroid carcinoma; ND: non diagnostic; Pts: patients.*

(CT peak ≥100 pg/ml) in only 6 patients (32%). The diameter of dominant nodule was 19.4±2.2 mm (range: 7-52; *p*=ns *vs* whole population and patients with normal basal CT values), with no significant difference among the 4 groups of patients. Twenty patients (87%) were euthyroid, 2 (8%) hypothyroid due to chronic thyroiditis, and 1 (5%) hyperthyroid due to a toxic adenoma. No patient had abnormal PTH, calcium, urinary metanephryne and catecholamine values. The results of FNAC are reported in Figure 1. A repeatedly non diagnostic cytological picture was found in 3 (13%) patients; a benign nodule was diagnosed in 6 (26%) subjects; FNAC was suspicious for malignancy (5 follicular neoplasm, 3 papillary thyroid carcinoma, 1 medullary thyroid carcinoma, 1 anaplastic thyroid carcinoma) in 10 (44%) patients, whereas it was diagnostic for malignancy (1 papillary thyroid carcinoma, 3 medullary thyroid carcinoma) in 4 (17%) patients. Although they had a benign cytology; patients N. 1, 7, 8, 9 and 14 were operated on because they were affected by a huge goiter with tracheal compression (patients N. 8 and 9 also complained of dysphagia).

The results of histological examination are reported in Figure 2. Histology disclosed a benign disease in 12 patients (52%) and a thyroid carcinoma in 11 patients (48%). Of the patients with benign thyroid diseases, a nodular goiter was diagnosed in 10 cases (43%), follicular adenoma in 1 case (4.5%) and Hurthle cell adenoma in 1 case (4.5%). Of the patients with malignancy, 2 (9%) had papillary thyroid carcinoma (PTC) and 9 (39%) medullary thyroid carcinoma.
Unilateral or bilateral CCH was observed in 8 out of 14 subjects (57%) with histologically undetected MTC.

Characteristics of 9 patients with MTC (Table 3)

MTC was histologically diagnosed in 9 patients, 0.6% of all patients with thyroid nodules, 21% of patients with thyroid malignancy, and 29% of patients with elevated basal serum CT concentration. These 9 patients were euthyroid and their age was 61.1±3.6 yr (range: 44-74; median: 61 yr; *p*=ns *vs* non-MTC patients), and the female to male ratio was 1:1. None of the patients (0%) belonged to group 1, 2 (22%) to group 2, 3 (33%) to group 3, 4 (45%) to group 4. Basal serum CT concentration was 507.2±198.2 pg/

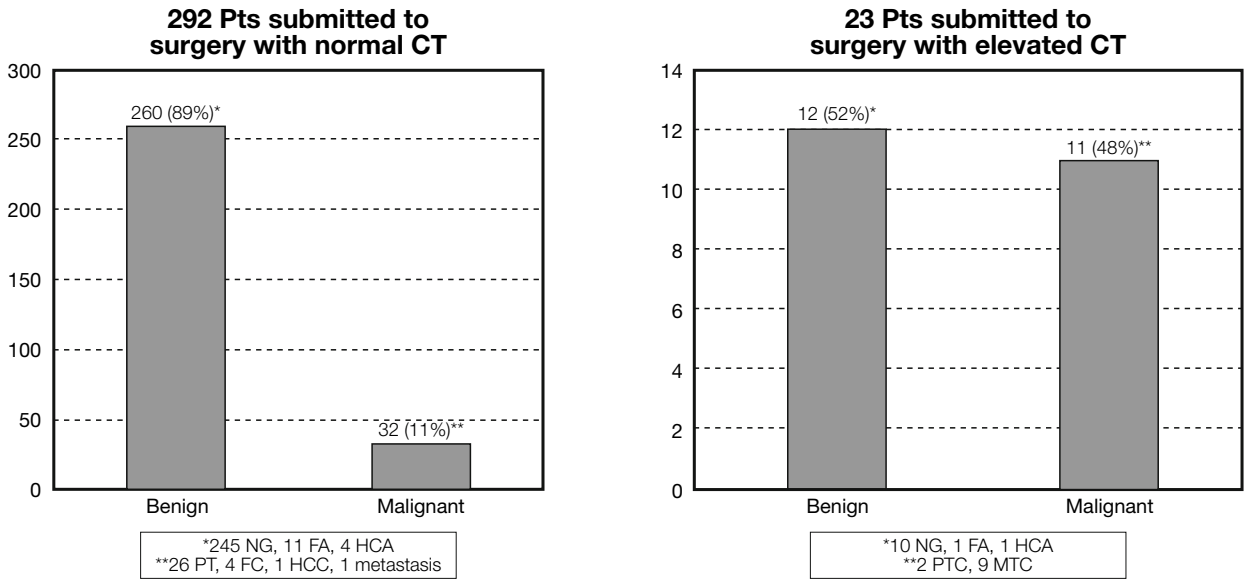

Fig. 2 - *Histology reports in patients with nodular goiter. CT: calcitonin; FA: follicular adenoma; HCA: Hurthle cell adenoma; HCC: Hurthle cell carcinoma; MTC: medullary thyroid carcinoma; NG: nodular goiter; PTC: papillary thyroid carcinoma; Pts: patients.*

ml (range: 15-1500; median: 80 pg/ml; $p<0.0001$ *vs* non-MTC patients). Nodule size was 20.9±4.7 mm (range: 7-44 mm; p=ns *vs* non-MTC patients). Ultrasound revealed a single nodule in 3 patients (33%) and multiple nodules in 6 patients (67%). Figure 1 summarizes the results of cytological examination. A repeatedly non diagnostic cytological picture was found in 2 (22.3%) patients; a benign nodule was diagnosed in 1 (11.1%) subject; FNAC was suspicious for malignancy (1 follicular neoplasm, 1 anaplastic thyroid carcinoma, 1 medullary thyroid carcinoma) in 3 (33.3%) patients, whereas it was diagnostic for medullary thyroid carcinoma in 3 (33.3%) patients. Histological examination showed a multifocal and unifocal carcinoma in 2 (22%) and 7 cases (78%), respectively. Neither capsular invasion nor extrathyroidal localization were found in 6 MTC cases (67%), whereas metastases to cervical lymph nodes were demonstrated in the remaining 3 MTC cases (33%) but no patient had distant metastases. Histological

Table 2 - *Results in 14 patients with elevated basal serum calcitonin (CT) concentrations submitted to thyroidectomy without a histological diagnosis of medullary thyroid carcinoma (MTC).*

Patients (no.)	Age (yr)/sex	Basal CT levels	Peak CT concentrations after Pg stimulation	Cytology	Histology
1	46/F	5.6	12	Benign	Nodular goiter
2	42/F	13	41	Suspicious for FN	ChTh+CCH-b
3	49/F	19	33	Suspicious for PTC	Hurthle cell FA+CCH-u
4	43/F	9	20	Suspicious for FN	FA
5	49/F	6.5	44	Suspicious for PTC	PTC+CCH-b
6	48/F	11	60	Bon-diagnostic	Nodular goiter+PTA+CCH-u
7	43/M	19	103	Benign	Nodular goiter+CCH-u
8	54/F	13	60	Benign	Nodular goiter+ChTh+CCH-b
9	53/M	14.5	49	Benign	Nodular goiter+ChTh
10	55/M	11	22	Suspicious for FN	Nodular goiter
11	57/F	18	39	Suspicious for PTC	Nodular goiter
12	28/F	17	64	Suspicious for FN	Nodular goiter+CCH-u
13	47/F	10.3	37	PTC	PTC+ChTh+CCH-b
14	45/F	8	15	Benign	Nodular goiter

CCH-b: bilateral C-Cell hyperplasia; CCH-u: unilateral C-cell-hyperplasia; ChTh: chronic thyroiditis; F: female; FA: follicular adenoma; FN: follicular neoplasm; M: male; Pg: pentagastrin; PTA: parathyroid adenoma; PTC: papillary thyroid carcinoma.

Table 3 - *Results of 9 patients with elevated basal serum calcitonin (CT) concentrations submitted to thyroidectomy and definitively diagnosed with medullary thyroid carcinoma (MTC).*

Patient	Age (yr)/sex	Group	Basal CT (pg/ml)	Pg-stimulated CT peak (pg/ml)	Cytology	Histology	TNM stage*
1	74/M	4	1230	–	Suspicious for ATC	28 mm MTC	III (T2N1M0)
2	72/F	4	1500	–	MTC	20 mm MTC + 1 mm PTC + bilateral CCH + ChTh	III (T2N1M0)
3	67/M	4	50	323	Non diagnostic	8 mm MTC	I (T1N0M0)
4	68/M	4	65	522	MTC	9 mm MTC+ChTh	I (T1N0M0)
5	60/F	3	1000	–	MTC	44 mm MTC + unilateral CCH	III (T3N1M0)
6	44/M	2	15	272	Benign	40 mm BN + 1 mm MTC + ChTh + bilateral CCH + 1 mm PTC	I (T1N0M0)
7	61/F	3	606	–	Suspicious for MTC	23 mm MTC + unilateral CCH	II (T2N0M0)
8	60/M	3	19	107	Suspicious for FN	7 mm MTC	I (T1N0M0)
9	44/F	2	80	1283	Non diagnostic	9 mm MTC	I (T1N0M0)

ATC: anaplastic thyroid carcinoma; CCH: C-cell hyperplasia; ChTh: chronic thyroiditis; FN: follicular neoplasm; Pg: pentagastrin; PTC: papillary thyroid carcinoma; T: tumor size; N: loco-regional lymph node involvement; M: distant metastases. *Based on the staging system recommended by the American Joint Committee on Cancer (AJCC) (27).

features consistent with CCH were found in 44% of MTC patients. Germline mutations of the *RET* proto-oncogene were absent in all MTC patients, indicating the sporadic nature of the tumor. After thyroidectomy, 6 out of 9 patients (67%) showed undetectable basal and Pg-stimulated serum CT concentration whereas the other 3 patients (33%), all classified as Stage III, had elevated basal serum CT concentration. All MTC patients are undergoing follow-up.

Sensitivity and specificity of basal and Pg-stimulated CT values, and FNAC

Sensitivity and specificity of both basal CT value and FNAC were calculated from the results obtained in the 315 patients submitted to surgery. Sensitivity of basal CT value and FNAC results in predicting MTC before surgery was 100 and 86%, respectively. FNAC was diagnostic, or suspicious, for MTC in all patients with histologically proven MTC nodules larger than 10 mm. On the contrary, a correct cytological diagnosis was obtained in only one out of five patients with micro-MTC nodules (ie, MTC nodules <10 mm in their largest diameter); in one patient with histologically proven microMTC, FNAC even demonstrated a benign lesion.
Specificity of basal CT value and FNAC results was 95 and 99%, respectively. The predictive values of positive and negative results of CT measurement were 39 and 100%, respectively. In all patients histologically diagnosed with thyroid disease other than MTC, basal CT values were <20 pg/ml (Table 2); on the contrary, all MTC patients had basal CT values ≥15 pg/ml (Table 3). Serum CT concentrations between 15 and 19 pg/ml were measured in patients with both benign thyroid diseases and MTC, whereas basal serum CT concentrations <15 pg/ml were measured only in patients with benign diseases. Basal CT values ≥20 pg/ml had a specificity of 100% in predicting MTC.
A CT value ≥100 pg/ml, 2 or 5 min following the administration of Pg, separated MTC from benign disease in all patients but one who had a peak CT value of 103 pg/ml. This patient proved to be definitely affected by nodular goiter and unilateral CCH. Thus, a Pg-stimulated CT value ≥100 pg/ml had a sensitivity of 100% and a specificity of 93%.

Results in patients with chronic autoimmune thyroiditis

Results in patients with chronic autoimmune (Hashimoto's) thyroiditis are summarized in Table 4.
Thirty-nine of all recruited 1425 patients had chronic thyroiditis. Seven of them (18%) showed elevated basal CT values, representing 30% of all patients with elevated basal CT values ($p<0.05$ *vs* patients with normal basal CT levels) and 33% of patients with MTC. Their age was 55.4±4.1 yr, 11 were males, 28 were females (female to male ratio of 2.5/1). In 34 of 39 patients, chronic thyroiditis was diagnosed in the presence of circulating TPO-Ab and/or TG-Ab, whereas in 5 patients it was diagnosed by histological examination. Fourteen of the 39 patients with chronic thyroiditis, 7 with elevated basal and 7 with normal basal CT values underwent surgery, and chronic thyroiditis was histologically confirmed in all cases. The 7 patients with normal basal CT values were operated on because of nodular goiter with compressive symptoms (5 cases) or suspicious cytology (2 cases). MTC was found in 3 out of 7 patients with both chronic thyroiditis and elevated basal CT concentration, and in none of the 7 patients with chronic thyroiditis and normal basal CT value. CCH was demonstrated in 5 out of 7 patients

Table 4 - *Results in 39 patients with chronic thyroiditis.*

	Patients with chronic thyroiditis and elevated CT	Patients with chronic thyroiditis and normal CT
Patients (no.)	7	32
Males/females	2/5	9/23
Age (yr)	54.3±4.4	55.8±3.9 (*p*=ns)
Basal CT values (pg/ml)	233±211.3	2.9±1 (*p*<0.02)
TG-Ab (mIU/l)	344±52	386±33 (*p*=ns)
TPO-Ab (mIU/l)	401±66	355±87 (*p*=ns)
TSH (microIU/ml)	6.1±2.4	7.3±3.3 (*p*=ns)

CT: calcitonin. TG-Ab: antibodies against thyroglobulin (normal range: 0-100 mIU/l). TPO-Ab: antibodies against thyro-peroxidase (normal range: 0-30 mIU/l).

with both chronic thyroiditis and elevated basal CT concentration, and in none of the 7 patients with chronic thyroiditis and normal basal CT value.

DISCUSSION

According to previous reports (17, 28-31), the present study indicates that CT measurement is more sensitive than FNAC in the pre-operative diagnosis of MTC, confirming the usefulness of routine CT measurement in all NTD patients, particularly those aged ≥40 yr old. In our series, the mean age of MTC patients averaged 61 yr. None of group 1 patients (ie, <40 yr old) was affected by MTC. The youngest subject with MTC was 44 yr old. MTC patients <40 yr have been occasionally reported. In their series of 1062 patients, Vierhapper et al. (31) diagnosed 6 MTC patients with a mean age of 60 yr and only one was 30 yr old. In 1167 patients, Niccoli et al. (30) observed 16 MTC patients with a mean age of 56 yr, but 3 were <40 yr. However, these authors did not perform genetic analysis to exclude MEN 2 syndrome in their MTC patients; indeed, it is well known that the familial forms of MTC occur earlier than their sporadic counterparts (32, 33).

Previous European studies (17, 28-31), recruiting large series of patients with NTD, demonstrated the possibility to diagnose MTC earlier with CT screening than FNAC. Hence, CT screening has been recommended in all patients with NTD (17, 28-31). However, some authors (22, 23), mainly in USA, argued against this statement because (1) CT test is not available in all centers; (2) Pg is not available at all in North America; (3) the costs of screening by CT test all patients with NTD are too high, as the incidence of thyroid nodules in the general population is elevated, falsely positive CT results frequently occur, and MTC is rare.

In our series, elevated (>5 pg/ml) basal CT values were observed in 23 out of 1425 (1.6%) patients affected by NTD, with or without MTC. This prevalence value is different from that reported by other studies, eg, it is higher than that found by Rieu et al. (0.8%) (29), but lower than that reported by Niccoli et al. (34/1167 subjects: 2.9%) (30) and Vierhapper et al. (55/1062: 5.2%) (31). Such different results may be due to patient selection and/or to different normal range values employed (34, 35). Indeed, previous studies used RIA or IRMA as laboratory methods to assess the ability of serum CT concentrations to predict unsuspected MTC in NTD patients before surgery (17, 28-31). To our knowledge, this study is the first employing the ILMA method, an immunoluminescence assay, to measure pre-operative CT levels in a large cohort of patients with NTD (Table 5). In comparison with RIA or unspecific IRMA, the ILMA is a highly specific method that recognizes the monomeric form of serum CT, whereas RIA also measures the precursors of CT (36). Engelbach et al. (36) demonstrated that the more specific determination of monomeric CT by the ILMA permits a more precise differentiation between post-operative normal and pathological values in thyroidectomized patients with and without MTC, and an earlier diagnosis of recurrent MTC. In the present work, we demonstrated that the ILMA also shows a high specificity in the pre-operative prediction of sporadic MTC.

Nodule size did not differ in patients with or without MTC and in those with or without hypercalcitoninemia. It is of interest that MTC patients with larger nodules showed higher basal CT values. A close correlation of tumor volume and pre-operative CT levels was recorded in MTC patients also by Sheuba et al. (37). This finding may be related to the greater CT secreting cellular mass that composes large nodules compared to small ones.

Out of 23 patients with elevated basal CT values, only 9 had MTC. This prevalence value (0.6%) of MTC in NTD patients is similar to that reported by other studies conducted in Italy (17, 28) and Austria (31). The

Table 5 - *Summary of results of previous studies investigating the impact of calcitonin (CT) measurement on the pre-operative diagnosis of medullary thyroid carcinoma (MTC) in patients with nodular goiter.*

Authors and Ref.	Yr	Patients enrolled	CT assay employed	CT assay: upper limit of normal range (pg/ml)	Prevalence of high CT levels (%)	Prevalence of MTC (%)
Pacini (27)	1994	1385	IRMA	20	0.5	0.6
Rieu (28)	1995	469	RIA-IRMA	10-35	0.8	0.8
Niccoli (29)	1997	1167	IRMA	10	2.9	1.4
Vierhapper (30)	1997	1062	RIA	5	5.2	0.6
Elisei (17)	2004	10,864	IRMA	20	0.4	0.4
Papi (present study)	2005	1425	ILMA	5	1.6	0.6

results of the two French studies performed by Rieu et al. (29) and Niccoli et al. (30) reported a prevalence of MTC in 0.8 and 1.4% of cases, respectively (Table 5). In our series, MTC represented 21% of all cases of thyroid carcinoma, an intermediate value between the MTC prevalence observed by Pacini et al. (15.7%) (28) and that reported by Rieu et al. (26.7%) (29).

In our patients, elevated serum CT concentrations were measured in all patients with MTC. A cut-off value of 20 pg/ml clearly separated patients with MTC from those with benign thyroid disease. The present and previous studies (17, 30, 31, 37) showed different cut-off values to discriminate benign from malignant C-cell disease. Basal CT values between 5 and 100 pg/ml actually represent the "gray-zone" where true and false positive cases overlap (17, 22, 23, 30, 31, 37). This wide range of overlapping results may be due to methodological differences in the CT assay making it difficult to compare different studies (35, 36). In accordance with our results, Pacini et al. (28) and Elisei et al. (17) reported a CT cut-off value of 20 pg/ml. In contrast, Niccoli et al. (30) showed a cut-off value >35 pg/ml, Scheuba et al. (37) >64 pg/ml, Vierhapper et al. (31) recommended a Pg test when basal CT exceeds 10 pg/ml (Table 5). In view of these findings, Pg-stimulated CT response is fundamental to correctly diagnose MTC when basal CT values are <100 pg/ml. In our series, all patients with both elevated basal serum CT concentrations (>5 pg/ml) and Pg-stimulated CT value >100 pg/ml had a histological diagnosis of MTC (100% sensitivity). However, a CT value of 103 pg/ml following Pg stimulation was observed in one patient with histological evidence of nodular CCH only. This form of CCH is considered by most authors as a true pre-neoplastic, ie, pre-malignant disorder that typically shows a greater response to Pg stimulus than the reactive or physiologic C-cell hyperplasias (38).

Our results showed that only 8 out of 14 patients (57%) with elevated basal CT values, and without histologically proven MTC, had CCH. In the other 43% of non-MTC patients elevated serum CT concentrations were not due to CCH. Benign nodules, differentiated thyroid cancer, non-thyroidal diseases including sepsis, renal failure, neuroendocrine tumors, and even exercise have been associated with increased serum CT concentrations (19-21, 39).

Interestingly, in our study elevated serum CT concentrations without MTC were observed in only one patient <40 yr. We observed a significant increase of mean basal CT values with advancing age. However, this finding was caused by the higher prevalence of MTC with aging. In our series, patients with elevated basal CT values had a significantly higher prevalence of chronic thyroiditis than those with normal CT values. Nonetheless, histological examination showed features of chronic thyroiditis in 3 out of 9 patients with MTC. An association between CCH and chronic thyroiditis has been previously reported (40, 41). Guyetant (40) reported the occurrence of CCH in 20% of patients with Hashimoto's thyroiditis. Recently, Karanikas et al. (18) reported autoimmune thyroiditis in 25% of hypercalcitoninemic patients both with and without NTD. In one case of this series, Hashimoto's thyroiditis coexisted with a micro-MTC, which was recognized only by pre-surgical CT measurement. In view of these findings, the authors proposed to extend the measurement of CT to patients with Hashimoto's thyroiditis. Niccoli et al. study (30) revealed that 50% of patients with MTC had histological features of thyroiditis. Other authors have also suggested a possible correlation between chronic thyroiditis, CCH and thyroid carcinoma (42, 43). At present, few data are available about the eventual etiologic relationship between chronic thyroiditis and MTC and further investigation is required on this topic.

In our series, FNAC identified only one third of MTC patients, and diagnosed as benign a nodule of a patient who had an abnormal basal CT value and a Pg-stimulated CT peak of 272 pg/ml. This patient had histologically proven MTC. In the remaining patients, FNAC was either non-diagnostic or suspicious for malignancy. On the contrary, FNAC had a specificity close to 100% in the diagnosis of MTC. Our results confirm previous reports (17, 28-31) showing the low sensibility and the high specificity of FNAC in the diagnosis of MTC.

The question whether a CT screening program is justified in all patients with NTD should be answered. A screening is justified when the screened disease is not rare, and its early detection affects the prognosis. MTC is the most aggressive of well-differentiated thyroid carcinomas, with a survival rate of 50% at 10 yr (1, 10). Since the prognosis relies on the stage of tumor at the time of diagnosis, and both conventional chemotherapy and radiotherapy are ineffective in curing advanced disease, early detection is crucial to perform the best surgical approach before extrathyroidal dissemination (1, 10). The Program of Community Action 1999-2003 of the European Union on rare diseases within the framework for action in the field of public health indicated that a disease is rare when its prevalence in the population is <5 per 10,000 (44). In our series, MTC had a prevalence of 0.6% of NTD cases. Pooling together the results of Italian, Austrian and French studies, the prevalence of MTC was 53 per 10,000 patients with NTD (28-31). Because the prevalence of thyroid nodules, detected by ultrasound, may approach 50% of general population (1, 3-5), MTC cannot be defined a rare disease, and we believe that NTD patients should be screened by basal CT measurement.

Nonetheless, the problem of costs deserves a special consideration. In the present study, we have identified a subgroup of patients, ie, NTD subjects >40 yr presenting with nodules <10 mm, who seem to be the best candidates to undergo the routine measurement of CT. Indeed, we found sporadic MTC only in patients >40 yr old; moreover, the FNAC failed to predict MTC in 50% of patients with micronodules. A further selection may be made sparing patients with a basal serum CT concentration ≥20 pg/ml the Pg stimulation test, because this cut-off value demonstrated a 100% sensitivity and specificity. In Italy, the total cost of a single basal CT measurement and a Pg stimulation test averages 45 and 180 Euros, respectively. If we had performed the basal and, when indicated, the Pg-stimulated CT test only to our >40 yr-old patients with micronodules (206 subjects altogether), we would have spent about 10,000 Euros. In contrast, if the basal CT measurement was not performed in our patients, 5 out of 9 patients histologically diagnosed with MTC would never be operated on, or would be submitted to an incomplete operation, or their operation would at least be delayed. We calculated that the total sum of 10,000 Euros we spent to screen this selected subgroup of patients by CT measurement is very close to the cost of additional tests (eg, annual basal and Pg-stimulated CT measurements; neck ultrasound examination; CT and/or PET whole body scans; radiotherapy) and surgical procedures, which a single patient should undergo when diagnosed or treated at a later stage, not achieving normalization of serum CT concentrations after surgery. Thus, in this perspective, the CT test seems not only cost-effective, but should even lead to a 400% saving of money.

In conclusion, the present study demonstrates that CT screening detects elevated CT values in 1.6% of NTD patients. Although CT is not a specific marker of MTC, its routine measurement represents a useful tool in the pre-operative evaluation of NTD patients, particularly those >40 yr presenting with nodules <10 mm, even when FNAC does not show malignant features.

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