Elevated calcitonin and procalcitonin levels in non-medullary benign and malignant thyroid nodules

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Summary

Background. **To evaluate the elevation rate of serum markers calcitonin (CT) and procalcitonin (ProCT) in patients with non-medullary benign and malignant thyroid nodules.**
Methods. **Before surgery CT and ProCT were measured in sera from 168 patients with histologically proven benign thyroid nodules (n=107) and non-medullary thyroid carcinomas (n=61). Sera from 112 apparently healthy blood donors were used as controls.**
Results. **Elevations in CT and ProCT levels were observed in 5 (2.9%) and 1 (0.5%) of 168 patients ($p<0.01$) with benign nodules, but non-medullary thyroid carcinomas.**
Conclusions. **Serum proCT showed a significantly lower false-positive rate than CT in patients with non-medullary benign and malignant nodules of the thyroid gland.**
Key-words: **medullary thyroid carcinoma, calcitonin, procalcitonin**

Introduction

Medullary thyroid carcinoma (MTC) is a malignant tumor of the thyroid C-cells, accounting for 5 to 10% of the thyroid tumours, and for 0.4 - 1.4% of all the thyroid nodules[1]. Serum calcitonin (CT) is a sensitive and specific marker for MTC and is used for the diagnosis and follow-up of MTC[2]. Multiple studies have shown that the routine measurement of serum calcitonin in patients with thyroid nodules is effective in the detection of clinically occult MTC[3-7]. One of the shortcomings of CT is that increases in serum marker concentrations might be observed in patients with non-thyroid diseases (i.e. small cell lung cancer, breast and pancreatic cancer, neuroendocrine tumors, renal failure) and patients with non-medullary thyroid carcinomas or benign thyroid diseases[8-10]. While different causes of hypercalcitoninemia can be effectively ruled-out by clinical history, an increase in serum CT is still challenging in patient presenting with a thyroid nodule. Pentagastrin stimulation test or through high-calcium perfusion improves the specificity of serum basal CT. Anyway, a tool to reduce stimulation and optimise cost-effectiveness of CT-based screening of thyroid nodules is advocated[11]. Procalcitonin (proCT), a CT-precursor, is found at low serum concentrations in healthy individuals while is known to increase in systemic inflammation, infection and sepsis[12] due to proCT production by non-thyroidal tissues[13]. Recently, proCT was proven to be as accurate as CT in MTC diagnosis and follow-up, with the advantages of better *in vitro* stability, lack of fragment/isoform interference, consistency of analytical methods and highly predictable half-life. However, proCT, but not CT, slightly increased in 3 of 55 patients with metastatic DTC and 1 patient with hyperthyroidism among 57 with benign thyroid diseases (i.e. hyperthyroidism, hypothyroidism and goiter and benign thyroid nodules), respectively[14]. Because neither hyperthyroidism nor metastatic DTC are typically screened by CT, the present study was undertaken to compare the specificity of serum CT and proCT in

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a large series of patients with non-medullary benign and malignant thyroid nodules (i.e. the typical setting of a CT-based MTC screening).

Materials and Methods

Patients

CT and ProCT was measured in the samples obtained before surgery from 168 patients with histologically confirmed benign thyroid nodules (n=107) and differentiated thyroid carcinoma (DTC) (n=61). Patients with history of small cell lung cancer, breast or pancreatic cancer, neuroendocrine tumors, sepsis, systemic inflammatory syndrome, renal failure and those taking proton-pump inhibitors were excluded. Sera from 112 apparently healthy blood donors were used as controls.

Methods

All patients underwent thyroid palpation, sonography, and measurement of thyrotropin (TSH), free T4 (fT4) and antithyroid peroxidase antibody (AbTPO) on Immulite 2000 platform (Siemens Healthcare Diagnostics, Deerfield, IL, USA). Thyroid scintigraphy and fine-needle aspiration citology (FNAC) were performed if needed, as further detailed.

Sonography

Thyroid sonography was performed in all patients using a Sonoline diagnostic ultrasound system (Siemens Healthcare Diagnostics, Erlangen, Germany) using a linear 7.5-MHz transducer by experienced physicians.

Ultrasound-guided fine-needle cytology (US-FNC)

Any hypoechoic nodule ≥ 5 mm with irregular margins and/or chaotic intranodular vascular spots and/or round or more tall than wide shape and/or microcalcifications was considered suspicious and US-guided FNAC performed as previously described[11]. Samples were evaluated by experienced cytopathologists and reported accordingly to British Thyroid Association guidelines[15].

Scintigraphy

All patients with a serum TSH above 0.1 mUI/L underwent thyroid scintigraphy. Planar acquisition was performed using a one-head γ-camera equipped with a dedicated low energy, high resolution, parallel hole collimator (ECAM, Siemens Healthcare Diagnostics, Erlangen, Germany). A planar image (anterior view) of the thyroid was obtained 20 min after i.v. administration of 74MBq Tc^{99m}-pertechnetate (matrix, 128 x 128 pixels; 600 seconds/image).

Surgery and surgical pathology

All patients underwent surgery due to suspicious FNAC (n=69), repeated non-diagnostic FNAC (n=12), hyperthyroidism (n=12), compressive symptoms (n=32), aesthetic (n=22) or other (n=21) reasons. Total thyroidectomy was performed in all cases with lymph node dissection along both recurrent nerves in cases with cytologically suspicious papillary thyroid carcinoma. Submitted thyroidectomy specimens were blocked entirely. On each block a hematoxylin/eosin stain was performed. Additionally, CT immunostains were made using the avidin-biotin-peroxidase method on specimens from patients with increased serum CT. C-cell hyperplasia was defined as >50 C-cells in a single low power field in both thyroid lobes[16].

Assays

Before thyroidectomy a blood sample was taken after a fasting overnight and serum was stored at –20 °C until assays for 1-5 days (median 2 days). CT was measured by an immunochemiluminometric assay using an Immulite 2000 platform. The assay has a functional sensitivity (lowest analyte concentration with an interassay coefficient of variation at 20%) of 5.0 pg/mL. ProCT was measured on a Kryptor system (BRAHMS, Berlin, Germany) by a homogenous time-resolved amplified cryptate emission immunometric fluorescent assay. The assay has a functional sensitivity of 0.1 ng/mL. Values greater than 2.0 ng/mL were automatically diluted by the instrument to obtain a value within the linear range of the assay. Algeciras-Schimnich et al recently reported in a cohort of 197 healthy subjects (98 males and 99 females) a reference upper limit for CT of 16.0 ng/mL for males and 8.0 ng/mL for females and for proCT of 0.15 ng/mL[14].

Statistical analysis

The Chi-square test (χ^2) was employed to compare the positive rate of each marker. A p-value < 0.05 was considered to be statistically significant.

Results

Demographic data and histological findings are summarized in the Table I. Elevations in CT and ProCT

Table I. Demographic data and histological diagnosis.

Patients	**168**
age	mean 54 yr, range 16–86 yr
sex	male 43 / female 125
Benign nodules	***107***
follicular adenoma	9
colloid goiter	54
hyperplastic goiter	26
lymphocytic thyroiditis	18
Thyroid carcinomas	***61***
papillary	54
follicular	7
lymph-node metastases	13
distant metastases	2
Controls	**112**
age	mean 34 yr, range 18–54 yr
sex	male 54 / female 58

Table II. CT and proCT positive rates in patients and controls.

	CT	ProCT	χ^2-*test* (p)
All patients (n=168)	**5**	**1**	**<0.01**
Benign nodules (n=107)	5	1	<0.01
Thyroid carcinomas (n=61)	0	0	-
Healthy controls (n=112)	0	0	-

were observed in 5 (2.9%) and 1 (0.5%) of 168 patients, respectively (p<0.01). None of patients with DTC and healthy controls had a false-positive CT or proCT result (Table II). Four of 5 patients with increased CT (included the only one with positive proCT) had nodular lymphocytic thyroiditis (Table III).

Discussion

Serum CT is a very sensitive marker for MTC but raised serum CT levels can be equally observed in other circumstances including non-thyroid and thyroid diseases[8-10,17]. In our series, a false positive serum CT was found in 4 patients with nodular lymphocytic thyroiditis and 1 with hyperplastic goiter while none of patients with DTC had an increased serum CT (overall false positive rate: 2.9%). Reactive C-cell hyperplasia (CCH) in neonates, elderly age, hyperparathyroidism, Hashimoto's thyroiditis, and follicular thyroid adenomas have been reported to increase serum CT concentration[16]. Typical cytological alterations would allow to detect the neoplastic CCH on hematoxylin/eosin sections, while the identification of the reactive type usually would require immunohistochemistry[18]. Inflammatory mediators and cytokines secreted by infiltrating lymphocytes in the thyroid parenchyma may be involved in reactive C-cell hyperplasia with CT secretion in these patients[19]. Accordingly, 3 and 1 of our 5 CT-positive patients, all with lymphocytic thyroiditis, displayed reactive CCH and borderline increased C-cell counts, respectively. Some authors proposed higher cut-off levels for basal CT to rule-out patients with reactive CCH from stimulation test but this approach is criticized by others[20,21]. Interestingly, only one patient had a slight increase in serum proCT in our series (0.5% false positive rate). Particularly, a negative proCT measurement could have been excluded 80% of CT-positive patients from stimulation tests and/or invasive diagnostic procedures in our series suggesting, in conclusion, that proCT is more specific than CT in patients presenting with non-medullary thyroid nodules.

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Table III. Histological diagnosis and marker levels in patients with positive CT and/or proCT.

	age	sex	Histology	IIC	CT (pg/mL)	ProCT (ng/mL)
1	46	F	Hyperplastic goiter	26	9.2	<0.1
2	42	F	Lymphocytic thyroiditis	65	13.8	0.20
3	23	M	Lymphocytic thyroiditis	92	19.2	<0.1
4	37	F	Lymphocytic thyroiditis	73	12.7	0.11
5	44	F	Lymphocytic thyroiditis	48	10.4	<0.1

Legend: IIC, immunohistochemistry (number of C-cells in a single low power field in both thyroid lobes).

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