



Efficacy of Procalcitonin Measurement in Patients after Total Thyroidectomy Due to Medullary Thyroid Carcinoma

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Abstract. Procalcitonin (PCT) is a protein synthesized by the thyroid C cells, inside which it is cut into calcitonin (CT) and calcitonin receptor-like receptor (CRLR). It remains undetectable in serum in normal conditions. Its level increases during inflammation and in small cell lung cancer. There have been studies suggesting that the PCT level increases in medullary thyroid carcinoma (MTC). So far there have been no reports that would assess the usefulness of PCT detection in MTC. Our aim was to evaluate the usefulness of serum PCT assays in patients with MTC. We investigated 24 patients at 17–78 years of age, all after total thyroidectomy due to MTC. All patients had serum CT concentrations measured by radioimmunoassay. The upper limit of the CT level was 60 pg/ml. The serum PCT was evaluated with an immunochromatographic kit. The reaction was considered positive when the PCT level exceeded 0.5 ng/ml. In all cases the C-reactive protein (CRP) serum level was measured. The statistical analysis was performed with Statistica 5.1G. The CT levels in all patients varied from 0 to 1410, mean 603.8 pg/ml. In 8 patients the CT level was within normal range, in 6 patients it was marginally, and in 10 patients markedly elevated. The PCT test was considered positive in 16 patients. There was correlation among serum PCT and CT concentrations (Spearman test, $p < 0.0001$). The PCT levels varied considerably among patients with normal, marginally and markedly elevated CT levels (Kruskal-Wallis test, $p = 0.0013$). All patients had normal CRP values. Fisher's exact test revealed a correlation between serum PCT and CT increase ($p = 0.04$). Further studies on a larger group of patients should be considered; thus, the PCT assay can be considered useful in cases of unclear CT concentration.

Key words: medullary thyroid carcinoma; procalcitonin; calcitonin; follow-up.

Introduction

Medullary thyroid carcinoma (MTC) is a rare malignancy of neuroectodermal origin that arises from parafollicular C cells of the thyroid gland. It constitutes 5–20% of all thyroid cancers. MTC is known to be a hormonally active neoplasm with a wide spectrum of secreted products, such as calcitonin (CT), calcitonin receptor-like receptor (CRLR),

CGRP, ACTH, serotonin, and VIP. To monitor the activity of a disease, the measurement of serum CT and CEA concentrations has been widely used^{9, 12}. The concentration of CT has also been evaluated as a screening of MTC in patients with a thyroid nodular goitre^{8, 13}.

CT, a 32 a peptide, is the end-product of enzymatic proteolysis of its origin protein, preprocalcitonin. Preprocalcitonin, the direct product of *CALC-1* gene trans-

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lation, is cut by endopeptidase in the endoplasmatic reticulum and forms procalcitonin (PCT; 13 kDa, a 116 aa protein). PCT then intracellularly disintegrates into CT and catacalcin. While, in physiological conditions, CT plays an important role in the calcium-phosphorus balance¹¹, PCT remains undetectable in serum and its physiological function has still not been recognized. Its serum concentration, however, increases considerably in cases of tissue systemic inflammatory response. Thus the monitoring of PCT concentration has been helpful in the diagnosis of patients with bacterial infections. Moreover, its increased concentration was noted in patients with small cell lung cancer and in complicated cases of myocardial infarction^{3, 7, 11, 14}. There have also been few studies supporting the view that an increased concentration of PCT can be found in patients with MTC. But, so far, there have been no reports in the literature that would clearly assess the usefulness of serum PCT detection in diagnosing or monitoring patients with MTC¹¹.

Therefore, the object of our study was to evaluate the usefulness of serum PCT evaluations in patients diagnosed with MTC, in whom a total thyroidectomy had been performed.

Materials and Methods

Twenty-four patients (17 women and 7 men) at ages of 17 to 78 years (mean 48.1 ± 14.7 years) were involved in the study. They all had had a total thyroidectomy performed due to MTC. The mean duration of disease since its diagnosis was 5.3 years. Diagnosis was based on the results of histopathological examination by means of immunocytochemical staining, with a positive reaction for CT. Two patients presented with distant metastases of MTC and 8 with a regional recurrence of the disease. Fourteen patients had remains of thyroid tissue disclosed by ultrasound investigation.

All patients had the serum CT concentration measured (with the use of a double antibody radioimmune assay, RIA-DPA). The upper range of normal for CT concentration was considered to be less than 60 pg/ml.

The concentration of PCT was evaluated in all our patients by the semiquantitative immunochromatographic sandwich method (PCT-Q kit, Brahms Diagnostica GmbH, Germany). The sandwich assay uses anti-catacalcin antibodies as a marker and anti-CT antibodies as a solid phase. It allows a detection of PCT at a wide range of concentrations: <0.5 ng/ml, 0.5–2.0 ng/ml, 2.0–10.0 ng/ml, and >10.0 ng/ml. The reaction was

considered positive with a PCT concentration equal to or greater than 0.5 ng/ml.

Since the half-life of PCT is only 24 h and, more importantly, of CT only a few minutes, a proper and quick preservation of blood samples is of a great importance. Thus, before the CT concentration is measured, its natural degradation must be avoided¹¹.

In our study the assessment of serum C-reactive protein (CRP) concentration was additionally performed (Turbiquant CRP kit, Dade Behring, Germany). Thereby, a misinterpretation of increased PCT concentrations due to the inflammatory response could be excluded. Values of more than 5 mg/l were considered positive.

The statistical evaluations were carried out with Statistica for Windows 5.1G software (StatSoft Inc., USA). Significance was considered as $p < 0.05$.

Results

Serum CT concentrations in all patients varied from 0 to 1410 pg/ml, with a mean of 507.2 ± 603.8 pg/ml. More specifically, 8 (33.3%) patients presented with a normal CT concentration, in 6 (25%) patients the CT concentration was marginally (<100 pg/ml) elevated and in 10 (41.7%) patients it considerably (>100 pg/ml) exceeded normal values (Fig. 1). PCT levels equal or greater than 0.5 ng/ml were found in 16 (66.7%) patients. Patient serum concentrations of CT, PCT and CRP are shown in Table 1. Moreover, there was a significant correlation between serum PCT and CT concentrations (Spearman ratio 0.84, $p < 0.0001$; Fig. 2). The PCT concentrations varied considerably among patients with normal, marginally and considerably elevated CT levels (Kruskal-Wallis ratio 13.3, $p = 0.0013$; Fig. 3). All patients presented with normal CRP values.

In 2 patients with distant metastases, the serum concentrations of CT and PCT were highly increased. In patients with local recurrence, the CT and PCT concentrations were increased. In the group of patients with residual thyroid tissue, the serum CT and PCT levels were undetectable in 4 individuals. In the next 4 individuals, CT levels were within the normal range (up to 60 pg/ml), while PCT concentrations were increased in 2 of them. In the remaining 6 patients with residual thyroid tissue, the CT concentrations were increased (up to 100 pg/ml) and the PCT levels were detectable in 4 of these patients.

The Fisher test revealed a significant correlation between serum PCT and CT increase ($p = 0.004$). The test sensitivity was 0.75, specificity 0.87, positive predictive value 0.75, and negative predictive value 0.87.

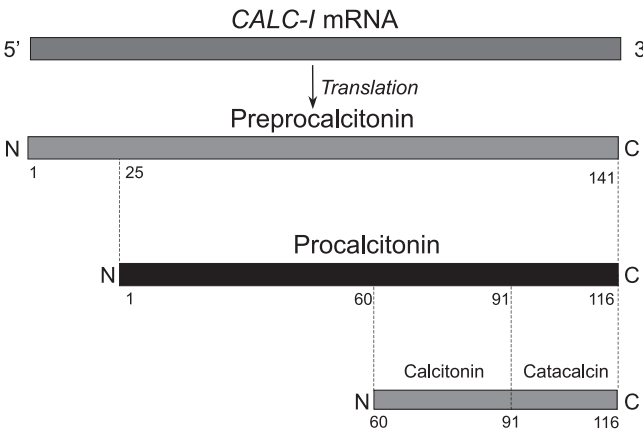


Fig. 1. Schematic diagram of protein and peptide products derived from *CALC-1* gene

Discussion

In our study we found a correlation between increased serum CT levels and the stage of the disease: in patients with considerably elevated CT concentrations, regional recurrence or metastases to regional lymph nodes were found. In two patients, distant metastases were detected. The RIA-DPC we used to assess the CT concentration is a method of low sensitivity compared with other, newer methods of CT detection (chemiluminescence, ELISA). It also allows detecting the origin proteins of CT, such as PCT^{5, 15, 16}. There is a possibility of false-positive results due to the occurrence of cross-reactions, for example with PCT, or the presence of heterophilic antibodies in serum. However,

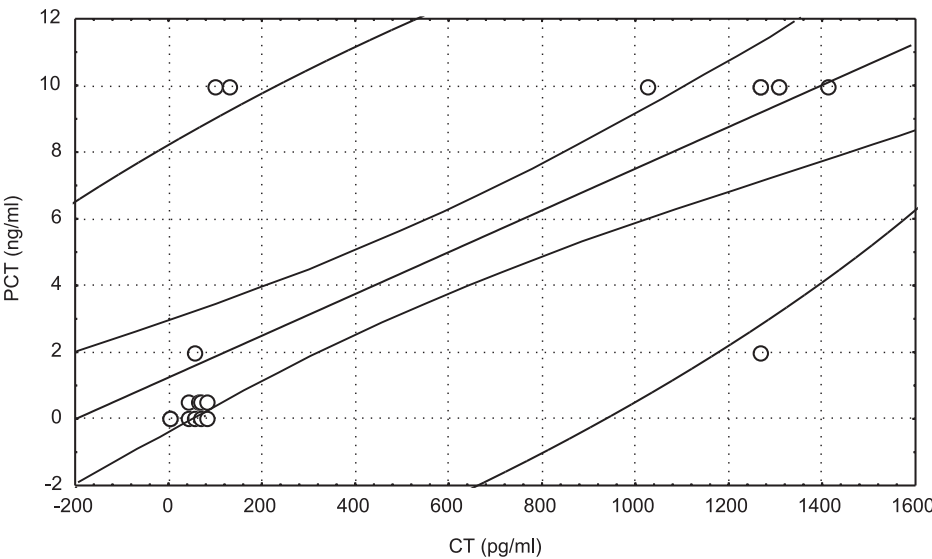


Fig. 2. Correlation between PCT and CT concentration in the studied group

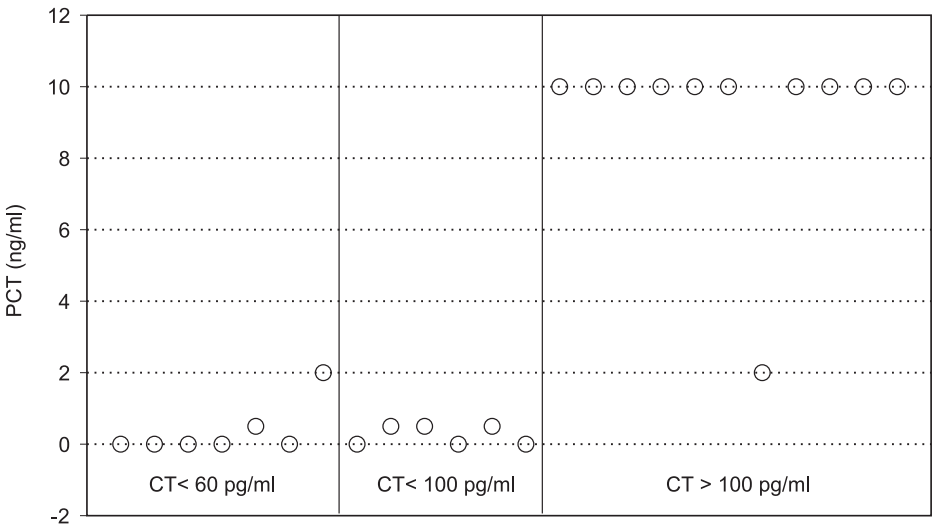


Fig. 3. Serum PCT concentrations in patients with normal, marginally and markedly elevated CT levels

Table 1. Results of CT, PCT and CRP assays in the studied group

	No.	Age (years)	Sex	CT (pg/ml)	PCT (ng/ml)	CRP (mg/l)
Patients with residual thyroid tissue	1	64	F	0	0	<5
	2	17	F	0	0	<5
	3	25	M	0	0	<5
	4	20	F	0	0	<5
	5	61	F	42	0.5	<5
	6	54	F	55	2	<5
	7	46	M	42	0	<5
	8	47	F	55	0	<5
	9	61	F	62	0.5	<5
	10	42	F	80	0.5	<5
	11	59	F	67	0.5	<5
	12	39	M	70	0	<5
	13	47	F	80	0	<5
	14	45	F	100	10	<5
Patients with local recidiva	15	78	F	130	10	<5
	16	51	F	1023	10	<5
	17	51	F	1268	2	<5
	18	44	F	1268	10	<5
	19	35	F	1268	10	<5
	20	43	M	1268	10	<5
	21	50	M	1268	10	<5
	22	55	M	1308	10	<5
Patients with metastases	23	73	M	1308	10	<5
	24	47	F	1410	10	<5

F – female, M – male.

in the group of patients we investigated, the severity of their condition was in high correlation with CT concentrations^{5, 15–17}.

PCT is a well-known marker that can be monitored in septic inflammatory disorders. However, even though MARUNA et al.¹¹ reports the possibility of hyperprocalcitoninemia in cases of MTC, no reports about the concentration of PCT in patients with MTC have so far been published.

In our study, in all 10 patients with considerably elevated CT concentrations (>100 pg/ml), PCT levels were also found to be increased. There were patients with distant metastases and local recurrence in this group of patients. This may reflect a considerably dysregulated process of CT synthesis or of posttranslative proteolysis in cases of MTC of high malignancy. It is widely known that PCT can be synthesized not only in a C cell of the thyroid gland, but also in many different types of cells. The production of PCT in cases of a septic inflammatory response can be the greatest example. The *CALC-1* genes can also be expressed in neuroendocrine cells of the respiratory tract, intestines, as well as the pituitary gland^{10, 11}. The possibility of cross reactions with CT during PCT detection can certainly be excluded, as the chromatographic method of PCT detection is based on the sandwich assay with the use of

anti-catacalcin antibodies, and catacalcin represent the C-end fragment of PCT, thus it never occurs in the structure of CT.

In the group of patients with marginally (<100 pg/ml) elevated CT concentration, 4 (60%) of them presented with a positive reaction for PCT. They had residual thyroid tissue after thyroidectomy with no signs of recurrence or metastases. Most probably, in the case of lower CT concentrations the process of its synthesis is less dysregulated and the process of origin protein posttranslative proteolysis is not disturbed.

We found it more difficult to interpret the results of PCT detection in the group of patients in whom CT levels stayed within normal values. These were patients with residual thyroid tissue after thyroidectomy. The PCT concentration exceeded normal values in 2 (25%) of these patients. To avoid a misinterpretation of increased PCT concentration in cases of septic inflammatory conditions, we also assessed the serum CRP concentration in such patients. The usefulness of comparing the correlation between PCT and CRP increase has been reported^{3, 7}. In all our patients the serum CRP concentration was found to be negative. We also excluded the presence of apparent recurrence or metastases of MTC. Nevertheless, persistent hypercalcitoninemia may occur in the case of recurrent disease of

an unknown location^{9, 12}. We also excluded all other disorders that may result in increased serum PCT concentration, such as renal insufficiency, complicated myocardial infarction or paraneoplastic synthesis of PCT in the case of lung cancer^{11, 14}. Moreover, in this group of patients the measurement of serum PCT concentration was conducted twice to avoid the Hook effect in CT detection^{1, 6}. This confirmed our previous results. One of the possible mechanisms of increased PCT concentration in normocalcitoninemic patients is the initial stage of MTC recurrence and, in such cases, the intensification of PCT secretion may precede the increase of serum CT concentration. In such patients, frequent measurements of CT levels might be required.

In our study we found a statistically important correlation between the increased concentrations of CT and PCT (Spearman ratio 0.84, with $p < 0.0001$). It was particularly noticeable in the group of patients with considerably elevated CT levels. Moreover, the Fisher test confirmed the correlation between serum PCT and CT elevation. The UICC recommendations for the specificity of neoplastic markers has a minimum of 0.9². Therefore, the results we obtained are not sufficient. They do not entitle us to introduce the PCT assessment to determine the progression of MTC in patients in whom total thyroidectomy was previously performed. All the other disorders that may cause a rise in PCT concentration must also be considered. It also seems reasonable to determine serum PCT levels whenever CT concentration seems to be increased for unclear reasons, thus to exclude cross-reactions between CT and PCT. The studies of D'HERBOMEZ et al.⁴ emphasize the need of PCT assessment every time the results of CT measurement are unclear. The authors noted the interference between CT and PCT detection in the case of the use of a sensitive immunometric assay of CT detection. In view of the high-positive predictive value that we obtained, the detection of PCT in a larger cohort of patients with MTC should be considered as well as the use of an assay of a newer generation for measuring the concentration of CT.

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