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Can procalcitonin be useful for medullary thyroid cancer?

Czy oznaczanie prokalcytoniny może być przydatne u chorych na rdzeniastego raka tarczycy?

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Abstract

Introduction: Calcitonin, the best known marker for medullary thyroid cancer (MTC), has several laboratory limitations which limit its use in the routines of non-specialized laboratories. Procalcitonin, the precursor of calcitonin, is free from these drawbacks.
The aim of this study was to compare calcitonin and procalcitonin levels in MTC patients with active disease or in remission, and in patients with non-toxic nodular goiter (NTNG).
Material and methods: Forty-three serum samples, obtained from 40 patients (6 MTC active disease patients, 23 MTC patients in remission, and 11 NTNG patients), were tested for calcitonin and procalcitonin levels. The levels of both markers were measured in 2 MTC patients with active disease before and after surgery. One was re-operated due to neck relapse, the other one due to liver metastases.
Results: Both procalcitonin and calcitonin levels were considerably higher in all MTC patients with the active disease. In two re-operated patients, the levels of both markers decreased after surgery but remained above the reference range. In the remission group of MTC patients, 18 had both markers within the reference range, 2 had slightly elevated calcitonin, and 3 patients exhibited both markers slightly increased. In the NTNG group, all but one patient had normal procalcitonin and calcitonin levels. Analysis revealed a significant correlation between procalcitonin and calcitonin levels ($r = 0.7383$; $p < 0.0001$).
Conclusions: Procalcitonin has a similar distribution of values as calcitonin and may be used for evaluation of MTC status in some situations when accurate CT estimation is not achievable. **(Pol J Endocrinol 2010; 61 (5): 430–436)**

Key words: *procalcitonin, calcitonin, medullary thyroid cancer, diagnostic marker*

Streszczenie

Wstęp: Kalcytonina jest najbardziej znanym i bardzo czułym markerem raka rdzeniastego tarczycy (MTC, *medullary thyroid cancer*), niemniej, jej oznaczanie nie należy do najłatwiejszych co jest wadą dla niewyspecjalizowanych laboratoriów. Prokalcytonina, prekursor kalcytoniny jest łatwiejsza w oznaczeniu. Celem pracy było porównanie stężeń kalcytoniny i prokalcytoniny u pacjentów z MTC wykazujących aktywną postać choroby lub będących w remisji oraz u pacjentów z wolem guzowatym nietoksycznym (NTNG, *non-toxic nodular goiter*).
Materiał i metody: Czterdzieści trzy próbki surowicy pobrano od 40 pacjentów (6 z aktywną postacią MTC, 23 z MTC w stadium remisji i 11 z NTNG) i zmierzono stężenia obu markerów. U 2 chorych z aktywnym MTC badania wykonano przed i po zabiegu operacyjnym. Jeden z nich był reoperowany z powodu wznowy na szyi, drugi miał przerzuty do wątroby.
Wyniki: Stężenia prokalcytoniny i kalcytoniny były znacznie podwyższone u wszystkich chorych z aktywną postacią MTC. U dwóch reoperowanych chorych stężenia obu markerów obniżyły się po operacji, ale utrzymywały się nadal powyżej normy. W grupie pacjentów w stadium remisji MTC 18 miało stężenie obu markerów w granicach normy, u 2 stwierdzono nieznacznie podwyższone stężenie kalcytoniny, a u 3 nieznacznie podwyższone stężenie obu markerów. W grupie chorych z NTNG, wszyscy chorzy z wyjątkiem jednego mieli normalne stężenia prokalcytoniny i kalcytoniny. Analiza statystyczna wykazała znamienną korelację stężeń prokalcytoniny i kalcytoniny ($r = 0{,}7383$; $p < 0{,}0001$).
Wnioski: Prokalcytonina ma podobny rozkład stężeń, co kalcytonina i obserwuje się znamienną korelację stężeń obu markerów. Może być ona wykorzystana do oceny chorych z MTC w sytuacjach kiedy stężenie CT jest niemożliwe do oznaczenia.
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Słowa kluczowe: *prokalcytonina, kalcytonina, rak rdzeniasty tarczycy, marker diagnostyczny*

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Introduction

Calcitonin (CT) is a 32 amino acid peptide hormone physiologically produced by C-cells in the thyroid gland. CT is the best known specific marker for medullary thyroid cancer (MTC). MTC is a relatively rare disease, accounting for 3–5% of thyroid cancers, but mortality is higher than with differentiated thyroid cancer. Baseline or stimulated CT levels of more than 100 ng/mL are observed in almost all cases of MTC [1]. As a general rule, patients who reach undetectable serum CT levels soon after surgery are those with the best prognosis [10, 11]. CT measurement is recommended in thyroid nodular goiter for early detection of MTC or C-cell hyperplasia [2–4].

Some limitations of CT use may be noted as increased CT concentration is also observed in other diseases such as renal failure, other neuroendocrine tumours, C-cell hyperplasia, some leukaemias, small cell carcinoma of the lung, breast cancer, pancreatic cancers, hyperparathyroidism, and autoimmune thyroiditis [5–9]. CT is rapidly broken down by serum proteases, which may lead to errors — false low or false negative results — if samples are not processed quickly [7]. Also, normalization of CT serum level after surgery does not always signal definitive cure of MTC [10, 11].

There are various (at least seven) immunoreactive isoforms of CT, which could give inaccurate results [12]. CT has a biphasic and concentration-dependent half-life at physiological and increased levels [6]. Additionally, dual site antibody-based immunoassays are commonly used in clinical laboratories to quantify the CT serum concentrations as a specific and sensitive marker of MTC. Heterophilic antibodies can interfere with these assays, leading to false results of CT levels [13]. A high-dose hook effect may give false low results for several tumour markers, such as PSA, CA 19-9, CA 125, and others [14]. This effect has also been reported for CT and could be responsible for false low results of calcitonin measurements [15, 16]. In contrast, some substances have been reported to produce false high CT levels. It has been demonstrated that high levels of vitamin C, urea, and creatine could cause false high levels of CT when measured by RIA [17].

The 116 amino acid precursor of CT, procalcitonin (PCT), is free from these limitations. It is a product of the CALC-I gene and consists of a centrally located calcitonin and two flanking peptides: N-terminal region (a 57 amino acid peptide) and katacalcin (a 21 amino acid peptide), connected by peptidyl glycine amidating mono-oxygenase [18]. In healthy people, regular enzymatic processing and further cleavage of PCT, occurring only in C-cells, produces mature CT [19]. In the presence of bacterial infection or sepsis, the extrathyroid transcription of the CALC-I gene is unblocked. An elevated plasma level of PCT is an early marker of bacterial and fungal infection or sepsis [20]. It is related to disease severity and inversely related to outcome and treatment response.

In contrast to CT, PCT has a concentration-independent half-life and excellent in vitro stability in serum or plasma [21, 22]. Renal secretion is not a major pathway of PCT elimination; therefore, renal failure does not affect the PCT levels [22]. PCT measurement is more available than CT measurement in many hospital laboratories.

Considering all these facts, we would like to evaluate PCT utility as a potential marker for MTC. For this aim, we compared calcitonin and procalcitonin levels in our MTC patients (with active disease or in remission) and in non-toxic nodular goiter patients (NNTG) as a control group.

Material and methods

Blood was collected aseptically by venipuncture, and serum was separated from the clot as soon as possible. The samples were stored frozen at -20^0C.

Forty-three serum samples were obtained from 40 patients. Data are shown in Table I.

Three groups of patients were enrolled in the study:

1. MTC active disease patients: (Table I, samples 1–9) n = 6, mean age 48.2; 5 females, 1 male. Four of them had disseminated disease (Table I, samples 6–9). PCT and CT levels were measured twice in one patient (PU) (Table I, samples 1 and 2): first — one day before the second operation, and next — three weeks after re-operation. She was re-operated due to the local neck recurrence. Liver metastases were excised in another patient (MA) with disseminated MTC (Table I, samples 3–5). This patient's blood was examined three times: the first time — the day before surgery, and then twice — two weeks and three months following resection of liver metastases.
2. Patients with MTC in remission: (Table I, samples 10–32) n = 23, mean age 56.1; 17 females and 6 males. Patients underwent radical surgery (resection R0) and were disease-free in clinical and imaging examinations. Their recent CT level remained within the reference range and was measured in an out-patient department.
3. NTNG patients whose diagnosis was confirmed by histopathology: (Table I, samples 33–43) n = 11, mean age 49.5; 10 females and 1 male.

All MTC patients underwent total thyroidectomy and lymphadenectomy. The final diagnosis was established by histopathology supported by immunohistochemistry (to detect the presence of CT). Lymph node dissection was performed in four compartments: cen-

Table I. *Patients involved in the study, CT and PCT levels, clinical diagnosis*
Tabela I. *Pacjenci zakwalifikowani do badania, stężenia CT i PCT, rozpoznanie kliniczne*

Patient	Sample (Nº)	Age (years)	Gender	PCT level [ng/mL]	CT level [pg/mL]	Clinical diagnosis
PU	1	47	Female	10.16	1708	MTC with lymph node metastases before operation
PU	2	47	Female	0.32	63.2	MTC with lymph node metastases after operation
MA	3	28	Female	6.67	2456	MTC before resection of liver metastases
MA	4	28	Female	2.92	995	MTC following resection of liver metastases: 2 weeks later
MA	5	28	Female	4.1	1441	MTC following resection of liver metastases: 3 months later
PB	6	49	Female	0.63	236	Disseminated MTC
MA	7	37	Female	5.52	2010	Disseminated MTC
CB	8	65	Female	0.76	159	Disseminated MTC
SZ	9	63	Male	1.48	238	Disseminated MTC
BJ	10	67	Female	< 0.1	13.3	MTC in remission
TG	11	63	Male	< 0.1	< 5	MTC in remission
JS	12	74	Female	< 0.1	< 5	MTC in remission
KB	13	57	Male	< 0.1	< 5	MTC in remission
SS	14	74	Male	< 0.1	< 5	MTC in remission
MM	15	29	Female	< 0.1	< 5	MTC in remission
SA	16	44	Female	< 0.1	< 5	MTC in remission
OB	17	39	Female	< 0.1	< 5	MTC in remission
OA	18	19	Female	< 0.1	< 5	MTC in remission
RU	19	76	Female	< 0.1	< 5	MTC in remission
WB	20	45	Female	< 0.1	< 5	MTC in remission
TL	21	65	Female	< 0.1	< 5	MTC in remission
BB	22	46	Female	< 0.1	< 5	MTC in remission
BM	23	62	Female	< 0.1	< 5	MTC in remission
GB	24	79	Female	< 0.1	7.7	MTC in remission
KJ	25	75	Male	< 0.1	< 5	MTC in remission
KJ	26	63	Male	< 0.1	< 5	MTC in remission
KA	27	36	Female	0.15	9.62	MTC in remission
AW	28	59	Male	0.16	40.6	MTC in remission
NR	29	37	Female	< 0.1	< 5	MTC in remission
SM	30	56	Female	< 0.1	< 5	MTC in remission
WJ	31	57	Female	0.27	21.9	MTC in remission
KK	32	69	Female	< 0.1	< 5	MTC in remission
WJ	33	42	Female	< 0.1	< 5	NTTG
MG	34	39	Female	< 0.1	< 5	NTNG
DK	35	29	Female	0.12	< 5	NTNG
MI	36	55	Female	< 0.1	< 5	NTNG
ŁW	37	66	Female	< 0.1	< 5	NTNG
KW	38	63	Male	< 0.1	< 5	NTNG
DE	39	47	Female	0.28	10.1	NTNG
HC	40	65	Female	< 0.1	< 5	NTNG
NZ	41	46	Female	< 0.1	< 5	NTNG
DA	42	46	Female	< 0.1	< 5	NTNG
NZ	43	46	Female	< 0.1	< 5	NTNG

Table II. *Age and CT, PCT levels in the studied groups*
Tabela II. *Wiek, stężenie CT i PCT w badanych grupach*

	Active MTC patients	MTC patients in remission	NTNG	p
Age	48 (IQR 37–63) (min.–max. 28–65)	59 (IQR 44–69) (min.–max. 19–79)	46 (IQR 42–63) (min.–max. 29–66)	0.3979
CT [pg/mL]	973 (IQR 236–2010) (min.–max. 159–2456)*	3.12 (IQR 1.27–7.70) (min.–max. 0.04–40.60)	2.66 (IQR 1.56–4.59) (min.–max. 0.12–10.10)	0.0005
PCT [ng/mL]	3.50 (IQR 0.76–6.67) (min.–max. 0.63–10.16)*	0.06 (IQR 0.03–0.08) (min.–max. 0.01–0.27)	0.06 (IQR 0.01–0.10) (min.–max. 0.00–0.28)	0.0005

*p ≤ 0.001 in comparison with all other groups. For both parameters, clear cut-off values with no overlap between groups could be established

tral, both lateral ones, and upper mediastinum. This approach was consistent with the guidelines of the Association of Polish Surgeons and the Polish Society of Oncological Surgery [23]. Post-operatively, all MTC patients received substitutive doses of levothyroxine to remain euthyroid and were followed up in our out-patient department.

All NTNG patients underwent total thyroidectomy without lymphadenectomy.

Patients with any signs of bacterial or fungal infection, renal failure, or other carcinomas were excluded from the study.

Laboratory examination

The frozen sera (–20°C) were taken to the laboratory unit and assayed for CT and PCT levels.

CT measurement

CT level was measured by the DiaSorin LIAISON® Calcitonin Assay (DiaSorin Inc- USA). It is a one-step sandwich chemiluminescence immunoassay (CLIA) intended for quantitative determination of CT in human serum. Affinity-purified mouse antibody to the synthetic human CT is coated to the solid phase. The second affinity-purified mouse antibody is conjugated to an isoluminol derivative. During incubation, CT binds to the solid phase and is subsequently bound by isoluminol conjugated antibody. Following incubation, the unbound material is removed with a wash cycle. The starter reagents are then added and a flash chemiluminescent reaction is initiated. The light signal is measured by a photomultiplier as relative light units (RLU) and is proportional to the concentration of CT present in calibrators, controls, or samples. CT reference range is 0–5.5 pg/mL for women and 0–18.9 pg/mL for men.

PCT measurement

PCT level was measured by LIAISON BRAHMS PCT Assay (DiaSorin S.p.A., Italy). It is a sandwich chemiluminescence immunoassay. A specific mouse monoclonal antibody is coated on magnetic particles (solid phase) and another monoclonal antibody is linked to an isoluminol derivative (isoluminol-antibody conjugate). During the incubation, PCT present in calibrators — samples or controls — binds to the solid phase monoclonal antibody, and then the antibody conjugate reacts with PCT already bound to the solid phase. After incubation, the unbound material is removed with a wash cycle. Subsequently, the starter reagents are added and a flash chemiluminescence reaction is thus induced. The light signal, and hence the amount of isoluminol-antibody conjugate, is measured by a photomultiplier as relative light units (RLU) and is indicative of PCT concentration present in calibrators — samples or controls.

PCT reference range is below 0.1 ng/mL.

Statistical analysis

Continuous variables are presented as medians and quartiles. Nonparametric Kruskall-Walis analysis of variance was used for inter-group comparisons with Bonferroni corrected Mann-Whitney's U test used for post-hoc evaluation. Spearman rank correlation test was used for correlation assessment. A p value of < 0.05 was chosen as statistical significance threshold.

Results

The three groups did not differ significantly with respect to age, but showed marked differences in both CT and PCT. Patients with active carcinoma showed markedly elevated levels of both markers, which were significantly higher than in patients in remission or the NTNG control group. Levels of analyzed markers within the studied groups are shown in Table II.

PCT and CT levels were higher in all active MTC patients — mean PCT 3.5 ng/mL and CT 973 pg/mL (Fig. 1 and 2).

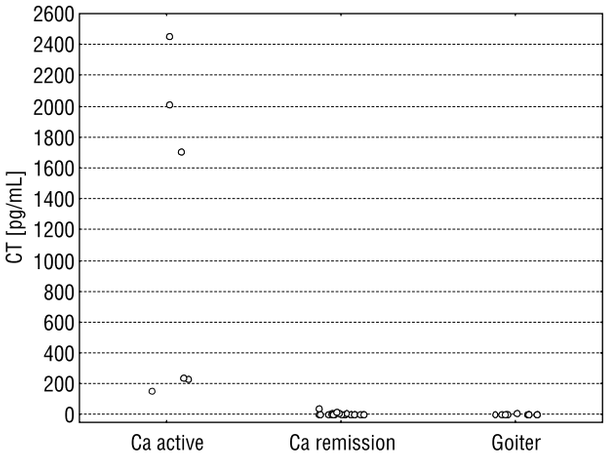

Figure 1. *CT levels in the studied groups*
Rycina 1. *Stężenie CT w badanych grupach*

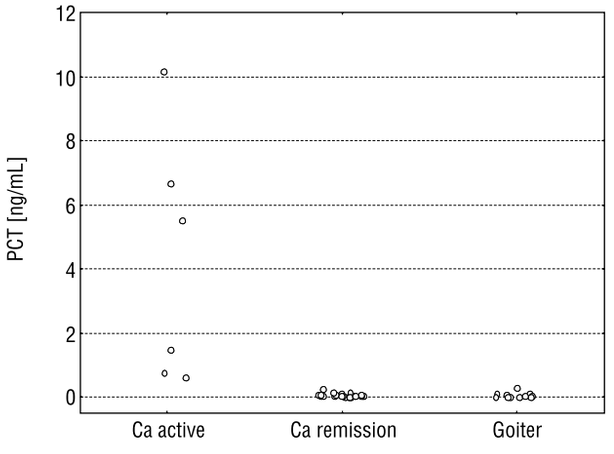

Figure 2. *PCT levels in the studied groups*
Rycina 2. *Stężenie PCT w badanych grupach*

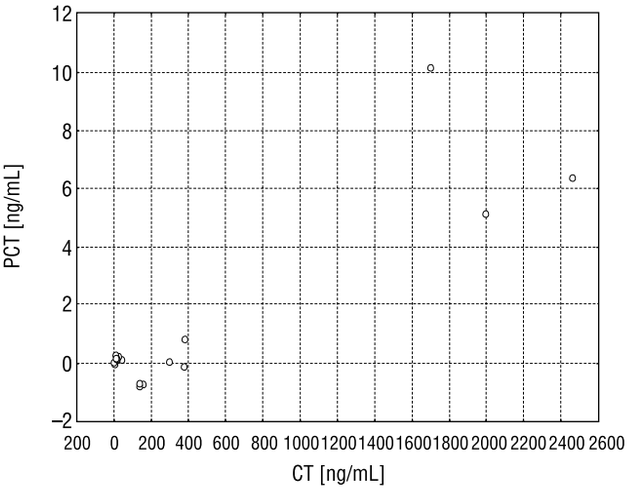

Figure 3. *Correlation of values of PCT and CT levels*
Rycina 3. *Korelacja wartości stężeń PCT i CT*

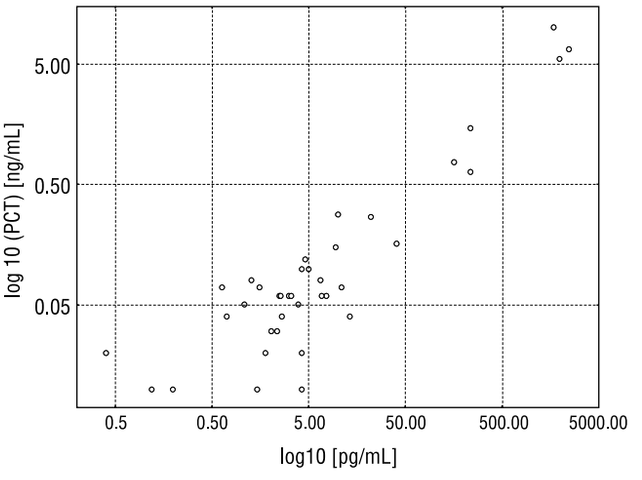

Figure 4. *Correlation of log-transformed values of PCT and CT levels*
Rycina 4. *Korelacja wartości stężeń PCT i CT (skala logarytmiczna)*

In one female patient (PU) (Table I, sample 1), PCT and CT levels were elevated (PCT — 10.16 ng/mL and CT — 1708 pg/mL). She was re-operated due to local neck recurrence; cervical exploration with four-compartment lymphadenectomy was performed. Three weeks after re-operation, PCT and CT and levels decreased to 0.32 ng/mL and to 63.2 pg/mL, respectively (Table I, sample 2). In another patient (MA) (Table I, sample 3), PCT level was 6.67 ng/mL and CT level was 2456 pg/mL. The patient had resectable liver metastases. Two weeks after surgically radical excision (resection R1), PCT and CT levels were still above the reference range, but were much lower. PCT level was 2.92 ng/mL and CT level was 995 pg/mL (Table I, sample 4). Three months after the operation clinical and imaging signs of relapse were observed. Relapse was confirmed by markedly elevated levels of both markers (Table I, sample 5). In four other patients with disseminated disease, both markers were significantly elevated (Table I, samples 6–9). In 18 MTC patients in remission, there was no increase in the levels of either marker. Another two patients from that group (BJ and GB) had slightly elevated CT levels (Table I, samples 10, 24). In three other patients (KA, AW and WJ), the levels of both markers were slightly increased (Table I, samples 27, 28, 31). In all (Table I, samples 33-43) but one patient (DE) (Table I, sample 39) in the NTNG group, PCT and CT levels were not elevated.

Correlation analysis showed a significant correlation between CT and PCT levels in the whole analyzed group ($r = 0.7383$; $p < 0.0001$; Fig. 3 and 4).

No correlations of either parameter with age were observed (R = –0.03; p = 0.86 for CT and R = –0.14; p = 0.39 for PCT). Neither CT nor PCT differed between males and females (p = 0.78 and 0.69, respectively).

Discussion

CT measurement is the gold standard for the diagnosis and follow-up of MTC. Some authors have even proposed that the CT level be checked before any thyroid operation to exclude or reveal MTC [24–26]. The greatest advantage of CT lies in a good correlation between clinical stage and CT level. However, as noticed earlier, CT measurement presents some inadequacies. In these cases some applications for PCT as an acceptable alternative may appear, especially as it is easier to be introduced in laboratories which do not perform many CT estimations. Its advantages include: a constant and predictable half-life, absence of isoforms, excellent in-vitro stability, and analytical consistency between assays [27, 28]. Under normal conditions, the plasma level of PCT is very low, but it is raised in bacterial and fungal infections and is, therefore, considered an inflammatory marker of non-thyroid origin. This study evaluates the usefulness of PCT as a potential MTC marker. To do this, we checked the correlation between clinical status and the levels of PCT and CT. There were four patients with disseminated MTC (inoperative disease) (Table I, samples 6–9). In this subgroup, both markers were substantially increased. PCT, like CT, properly reflected MTC dissemination in a female patient (PU) (Table I, samples 1–2); both PCT and CT levels were elevated. This patient was re-operated due to local neck recurrence, and extensive cervical exploration with four-compartment lymphadenectomy was performed. A reduction in the levels of both markers was observed three weeks later. In another patient (MA) (Table I, samples 3–5), computed tomography revealed metastases located in the left lobe of the liver. R1 resection was performed. As in the previous patient, the levels of both markers decreased post-operatively. Three months later, both CT and PCT levels were checked once again. Both markers were considerably increased, and presently further investigation of a new metastatic site is in progress.

In disseminated MTC patients, PCT reflected the active stage of the disease with similar precision as did CT. Our results were consistent with the findings of Matzaraki et al. [29], who demonstrated that PCT levels were markedly elevated in cancer patients with generalized metastatic disease. Twenty-three MTC patients in post-operative remission were enrolled in our study. They revealed no metastases in clinical and imaging examinations, and the most recent CT levels measured in the out-patient department were not increased. PCT and CT levels, measured for the study, were not increased in 18 patients, while PCT alone was not increased in 20 patients. This suggests that PCT could be used as a marker in the follow-up of MTC patients.

Eleven patients operated for NTNG were included in the study as a control group. We found no increase in the levels of PCT and CT in these patients, except slightly elevated PCT and CT levels in one patient (DE) before routine thyroidectomy (Table I, sample 39). This suggests that PCT is specific enough to serve as a marker in MTC patients.

Conclusions

Procalcitonin has a similar distribution of values as calcitonin may be used for evaluation of MTC status in some situations when accurate CT estimation is not achievable.

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