Calcitonin and Procalcitonin in Patients with Medullary Thyroid Cancer or Bacterial Infection*

Kalcytonina i prokalcytonina u pacjentów z rakiem rdzeniastym tarczycy lub infekcją bakteryjną

Abstract

Objectives. To evaluate procalcitonin (PCT) utility as a marker of medullary thyroid cancer (MTC).

Material and Methods. Calcitonin (CT) and PCT levels were measured in MTC patients and patients with serious bacterial infections. 70 patients were enrolled in the study: 6 MTC active patients: 4 with disseminated, unresectable disease and 2 re-operated patients, in whom markers were checked before and after surgery; 23 MTC patients in remission after radical surgery; 11 non-toxic nodular goiter (NTNG) patients; 30 patients with severe, bacterial infection or sepsis.

Results. All MTC active patients had greatly elevated CT and PCT levels. In two re-operated patients, marker levels decreased but were still above the reference range. In 15 MTC patients in remission, the levels of either marker were not increased. Both markers were slightly increased in 3 patients in this group, while CT was elevated in 5 patients. In all but 1 patient in the NTNG group, both marker levels were not elevated. Among patients with bacterial infection, PCT and CT levels showed no increase in 8 patients, both markers were elevated in 10 patients, and an increase of PCT levels was seen in 10 patients while of CT only in 2 patients. Correlations between CT and PCT values were very strong in MTC patients (r = 0.95; p = 0.004 for active MTC, r = 0.60; p = 0.002 for MTC patients in remission) and in patients with NTNG (r = 0.77; p = 0.02). In patients with infection, both parameters were completely independent (r = 0.002; p = 0.99).

Conclusions. PCT measurement could be an alternative to CT measurement for evaluation of MTC status (Adv Clin Exp Med 2012, 21, 2, 169–178).

Key words: procalcitonin, calcitonin, medullary thyroid cancer.

Streszczenie

Cel pracy. Ocena przydatności prokalcytoniny jako markera raka rdzeniastego tarczycy.

Materiał i metody. Stężenia kalcytoniny i prokalcytoniny zmierzono u pacjentów z rakiem rdzeniastym tarczycy lub ciężką infekcją bakteryjną. Do badania włączono 70 pacjentów: 6 pacjentów z aktywną postacią raka rdzeniastego tarczycy: 4 z rozsianą, nieresekcyjną postacią nowotworu; u 2 pozostałych chorych stężenia markerów zmierzano przed i po operacji; 23 pacjentów z rakiem rdzeniastym tarczycy w remisji po radykalnym zabiegu operacyjnym; 11 pacjentów z wolem guzowatym obojętnym; 30 pacjentów z ciężką infekcją bakteryjną lub posocznicą.

 Wyniki. Wszyscy pacjenccy z aktywną postacią raka rdzeniastego tarczycy mieli znacznie zwiększone stężenia kalcytoniny i prokalcytoniny. U dwóch reoperowanych chorych stężenia obu markerów zmniejszyły się, ale wciąż pozostawały powyżej normy. U 15 pacjentów w remisji raka rdzeniastego tarczycy nie odnotowano zwiększenia stężenia żadnego z markerów. Stężenia obu markerów były nieznacznie zwiększone u 3 chorych z tej grupy. U 5 pacjentów odnotowano jedynie nieznacznie zwiększone stężenie kalcytoniny. U wszystkich oprócz jednego

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Calcitonin (CT) is a 32-amino acid peptide physiologically generated mainly from the thyroid C-cells [1]. It is formed by the proteolytic cleavage of a larger prepropeptide, which is the product of the CALC1 gene. The CALC1 gene belongs to a superfamily of related protein hormone precursors including islet amyloid precursor protein, calcitonin gene-related peptide, and the precursor of adrenomedullin. Calcitonin has a mild and transient hypocalcemic effect, and it was originally thought to play an important role in skeletal homeostasis. This effect is mediated by the interaction of calcitonin with a specific receptor on the osteoclast. In almost all of the in vitro and in vivo studies conducted during the past 40 years, researchers concluded that CT is a basic inhibitor of bone reabsorption and its activity is modulated by several activators of osteoclastic function including parathyroid hormone (PTH). Calcitonin is considered to be a sensitive and specific marker for medullary thyroid cancer (MTC). MTC is a relatively rare disease, accounting for 3–5% of thyroid cancers, but MTC mortality is higher than that of differentiated thyroid cancer. Baseline or stimulated CT levels of more than 100 ng/ml are observed in almost all cases of MTC [2]. As a general rule, patients who reach undetectable serum CT levels soon after surgery are those with the best prognosis [3]. Routine calcitonin measurement has therefore been recommended in the diagnostic evaluation of patients with nodular thyroid disease by some authors to exclude MTC [4, 5]. CT as a laboratory marker has some limitations. Modest increases in serum CT concentrations might be revealed in other diseases such as: C cell hyperplasia, other neuroendocrine tumors, certain leukemias, systemic mastocytosis, small cell carcinoma of the lung, breast or pancreatic cancer, renal failure, hyperparathyroidism, autoimmune thyroiditis, pregnancy, lactation, and during the neonatal period [5–8]. CT has a concentration-dependent and biphasic half-life of 15 and 40 min at physiological concentrations; and 3 and 30 h at increased concentrations. CT is rapidly broken down by serum proteases, which may lead to error – false low or false negative results, if samples are not processed expeditiously after a blood draw. Patients who reach undetectable serum CT levels soon after surgery are those with the best prognosis, however normalization of serum CT after surgery is not an index of definitive cure in MTC [3]. The pattern of serum heterogeneity of patients with medullary thyroid cancer is characterized by the presence of at least seven different fractions of immunoreactive calcitonin. Furthermore, 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 and cause erroneous results [9]. A high-dose hook effect may give falsely low results for several tumor markers, such as PSA, CA 19-9, CA 125 and others (10). This effect has also been described in CT measurements, and it could lead to falsely low results [11, 12]. On the contrary, high levels of some substances: vitamin C, urea and creatine could cause falsely high levels of CT measured by RIA [13].

Procalcitonin (PCT), a 116-amino acid peptide, is also produced by C-cells in the thyroid and its concentration in the serum of healthy individuals is very low (< 0.1 ng/mL) [14]). It is a product of the CALC-I gene [15]. Physiologically, the extrathyroid transcription of the CALC-I gene is blocked. Microbial infections increase CALC-I gene expression, thus leading to a release of PCT. During microbial infection, there is an increase of CALC-I gene expression which causes a release of PCT from all parenchymal tissues and differentiated cell types throughout the body, including the liver and peripheral blood mononuclear cells [16]. The inflammatory release of PCT can be induced in two main ways: by toxins released by microbes (endotoxin); or through proinflammatory cytokines released in a cell-mediated host response (e.g. interleukins 1b and 6, tumor necrosis factor-alpha) [16]. Nowadays, PCT is known as a biomarker of bacterial or fungal infection and sepsis.

In contrast to CT, PCT has a concentration-independent half-life and excellent in vitro stability in serum or plasma [17]. Renal secretion is not a major pathway of PCT elimination, therefore renal failure does not affect the PCT levels significantly.
PCT measurement is more available and affordable than CT measurement in many hospital laboratories [18]. Considering all these facts, the authors would like to assess PCT utility as a potential marker for MTC. To do this, they measured CT and PCT levels in four groups of patients: 1) MTC patients with active disease; 2) MTC patients in remission; 3) non-toxic nodular goiter (NTNG) patients; 4) patients with clinically- and laboratory-confirmed bacterial infection.

**Material and Methods**

Blood samples were taken aseptically by venipuncture and serum was separated from the clot as soon as possible. The samples were stored frozen at –20°C.

Seventy-three serum samples were obtained from seventy patients.

The patients enrolled in the study were divided into four groups.

**Group 1**

6 MTC patients with active disease (Table 1), mean age 48.2, 5 females, 1 male.

PCT and CT were measured once in 4 patients. They had disseminated disease that made re-operation impossible. In one patient (PU) (Table 1), CT and PCT levels were measured twice: first, one day before the second operation and next, three weeks after re-operation. She was re-operated on due to local neck recurrence. Liver metastases were excised in the last patient (MA), who unlike the others had disseminated MTC (Table 1). This patient’s blood was examined three times: first, the day before surgery and then twice, two weeks and three months following the resection of liver metastases.

**Group 2**

There were 23 MTC patients in remission (Table 2), mean age 56.1, 17 females and 6 males. These patients underwent radical surgery (resection r0) – total thyroidectomy and lymphadenectomy. Lymph node dissection was performed in four compartments – central, both lateral and upper mediastinal. This approach was consistent with the guidelines of the Association of Polish Surgeons and the Polish Society of Oncological Surgery. The final diagnosis was established by histopathology supported by immunohistochemistry (to detect the presence of CT). The patients were disease-free in clinical and imaging examinations. Their last CT level was measured in an out-patient department and it remained within the reference range. Post-operatively, all MTC patients received substitutive doses of levothyroxine to remain euthyroid and were followed up in authors’ out-patient department.

**Group 3**

There were 11 NTNG patients (Table 3), mean age 49.5, 10 females and 1 male, whose diagnosis was confirmed by histopathology. All NTNG patients underwent total thyroidectomy without lymphadenectomy. The patients from groups 1–3 had no bacterial or fungal infection, renal failure or other carcinomas.

**Group 4**

There were 30 patients (Table 4) with severe, bacterial infection or sepsis, mean age 59.5, 11 females and 19 males.

**Laboratory Examination**

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

**CT Measurement**

The CT level was measured using the DiaSorin LIAISON Calcitonin Assay (DiaSorin Inc., USA). This assay is a one-step sandwich chemiluminescence immunoassay (CLIA) intended for the quantitative determination of CT in human serum. An affinity-purified mouse antibody to the synthetic human CT is coated to a solid phase. Liver metastases were excised in the last patient (MA), who unlike the others had disseminated MTC (Table 1). This patient’s blood was examined three times: first, the day before surgery and then twice, two weeks and three months following the resection of liver metastases.

**PCT Measurement**

The PCT level was measured using the Liaison Brahms PCT Assay (DiaSorin S.p.A., Italy). This assay is a sandwich chemiluminescence immunoassay. A specific mouse monoclonal antibody is coated on magnetic particles (solid phase); another monoclonal antibody is linked to an isoluminol derivative.
(isoluminol-antibody conjugate). During incubation, the PCT present in the calibrators – samples or controls, binds to the monoclonal antibody in the solid phase and subsequently the antibody conjugate reacts with PCT already bound to the solid phase. After incubation, the unbound material is removed in 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.

The PCT reference range is below 0.1 ng/ml.

**Statistical Analysis**

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

**Results**

The four groups did not differ significantly with respect to age, but showed marked differences in both CT and PCT levels.

PCT and CT levels were higher in all active MTC patients – mean PCT 3.5 ng/ml and mean CT 973 pg/ml (Tables 1 and 2).

In patient (PU) (Table 1), PCT and CT levels were markedly increased (PCT 10.16 ng/ml and CT 1708 pg/ml). She was re-operated due to the local neck relapse. Cervical exploration and four-compartment lymphadenectomy was performed. Three weeks after re-operation, PCT and CT levels had decreased to 0.32 ng/ml and 63.2 pg/ml, respectively (Table 1).

In patient (MA) (Table 1), the PCT level was 6.67 ng/ml and the CT level was 2456 pg/ml. The patient had resectable liver metastases. Two weeks after radical surgical excision (resection R1), PCT and CT levels were much lower but still above the reference range. PCT was 2.92 ng/ml and CT was 995 pg/ml (Table 1). Three months after the operation, clinical and imaging signs of relapse were observed. Relapse was confirmed by markedly elevated levels of both markers – the PCT level was 4.1 ng/ml, and the CT level was 1441 pg/ml (Table 1). In four other patients with active disseminated disease, both markers were significantly elevated (Table 1).

In MTC patients in remission, the mean PCT level was 0.06 ng/ml and the mean CT level was 3.12 ng/ml (Table 2). In 15 patients from this group, neither of the markers was elevated (Table 3). Five patients from this group (GB, BJ, WB, TL and BM) only had elevated CT levels (Table 3), while in three (KA, AW and WJ) (Table 3), the levels of both markers were slightly increased (Table 3).

In the NTNG group, PCT and CT levels were not elevated in all but one patient (DE) (Table 4).

In the group of patients with bacterial infection, the mean PCT level was 0.4 ng/ml and the mean CT level was 4.1 pg/ml (Table 2). The PCT and CT lev-

<table>
<thead>
<tr>
<th>Table 1. The MTC active patients involved in the study, CT and PCT levels, clinical diagnosis</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Patient</strong></td>
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<tr>
<td>-----------</td>
</tr>
<tr>
<td>PU</td>
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<tr>
<td></td>
</tr>
<tr>
<td>MA</td>
</tr>
<tr>
<td></td>
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<tr>
<td></td>
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<tr>
<td>PB</td>
</tr>
<tr>
<td>MA</td>
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<tr>
<td>CB</td>
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<tr>
<td>SZ</td>
</tr>
</tbody>
</table>
els were not increased in 8 patients from this group (Table 5). Both markers were elevated in 10 patients. While an increase in only PCT or CT levels were seen in 10 and 2 patients respectively (Table 5).

Levels of CT and PCT are shown in the Figures 1 and 2, respectively.

The statistical analysis shows that correlations between CT and PCT values were very strong and significant in individuals without infection – $r = 0.95$; $p = 0.004$ in patients with active MTC, $r = 0.60$; $p = 0.002$ in MTC patients in remission, $r = 0.77$; $p = 0.02$ in patients with NTNG. In individuals with inflammation, both parameters were completely independent – $r = 0.002$; $p = 0.99$.

The authors concluded that PCT shows a similar distribution of values to CT and strongly correlates with CT levels in the patients without infection. Greatly elevated PCT levels in patients with active MTC or infection in contrast to both individuals in remission and NTNG offers potential for using PCT as a diagnostic marker of either condition, while CT values can only be used to ascertain active MTC status.

### Table 2. Levels of analyzed markers within the studied groups

<table>
<thead>
<tr>
<th>Patient category</th>
<th>Mean (IQR)</th>
<th>Min–Max</th>
</tr>
</thead>
<tbody>
<tr>
<td>MTC active patients</td>
<td>973 (IQR 236–2010)</td>
<td>(min-max 159–2456)</td>
</tr>
<tr>
<td>MTC patients in remission</td>
<td>973 (IQR 1.27–7.70)</td>
<td>(min-max 0.04–40.60)</td>
</tr>
<tr>
<td>NTNG patients</td>
<td>2.66 (IQR 1.56–4.59)</td>
<td>(min-max 0.12–10.10)</td>
</tr>
<tr>
<td>Patients with inflammation</td>
<td>4.10 (IQR 0.87–7.50)</td>
<td>(min-max 0.06–19.80)</td>
</tr>
</tbody>
</table>

### Table 3. The MTC patients in remission involved in the study, CT and PCT levels, clinical diagnosis

<table>
<thead>
<tr>
<th>Patient (Pacjent)</th>
<th>Age – years (Wiek – lata)</th>
<th>Gender (Płeć)</th>
<th>PCT level (ng/ml) (Stężenie PCT)</th>
<th>CT level (pg/ml) (Stężenie CT)</th>
<th>Clinical diagnosis (rozpoznanie)</th>
</tr>
</thead>
<tbody>
<tr>
<td>GB</td>
<td>79</td>
<td>female</td>
<td>&lt; 0.1</td>
<td>7.7</td>
<td>MTC in remission</td>
</tr>
<tr>
<td>KJ</td>
<td>75</td>
<td>male</td>
<td>&lt; 0.1</td>
<td>&lt; 5</td>
<td>MTC in remission</td>
</tr>
<tr>
<td>BJ</td>
<td>67</td>
<td>female</td>
<td>&lt; 0.1</td>
<td>13.3</td>
<td>MTC in remission</td>
</tr>
<tr>
<td>GB</td>
<td>62</td>
<td>male</td>
<td>&lt; 0.1</td>
<td>&lt; 5</td>
<td>MTC in remission</td>
</tr>
<tr>
<td>SJ</td>
<td>74</td>
<td>female</td>
<td>&lt; 0.1</td>
<td>&lt; 5</td>
<td>MTC in remission</td>
</tr>
<tr>
<td>KB</td>
<td>57</td>
<td>male</td>
<td>&lt; 0.1</td>
<td>&lt; 5</td>
<td>MTC in remission</td>
</tr>
<tr>
<td>SS</td>
<td>74</td>
<td>male</td>
<td>&lt; 0.1</td>
<td>&lt; 5</td>
<td>MTC in remission</td>
</tr>
<tr>
<td>MM</td>
<td>29</td>
<td>female</td>
<td>&lt; 0.1</td>
<td>&lt; 5</td>
<td>MTC in remission</td>
</tr>
<tr>
<td>SA</td>
<td>44</td>
<td>female</td>
<td>&lt; 0.1</td>
<td>&lt; 5</td>
<td>MTC in remission</td>
</tr>
<tr>
<td>OB</td>
<td>39</td>
<td>female</td>
<td>&lt; 0.1</td>
<td>&lt; 5</td>
<td>MTC in remission</td>
</tr>
<tr>
<td>OA</td>
<td>19</td>
<td>female</td>
<td>&lt; 0.1</td>
<td>&lt; 5</td>
<td>MTC in remission</td>
</tr>
<tr>
<td>RU</td>
<td>76</td>
<td>female</td>
<td>&lt; 0.1</td>
<td>&lt; 5</td>
<td>MTC in remission</td>
</tr>
<tr>
<td>WB</td>
<td>45</td>
<td>female</td>
<td>&lt; 0.1</td>
<td>6.6</td>
<td>MTC in remission</td>
</tr>
<tr>
<td>TL</td>
<td>65</td>
<td>female</td>
<td>&lt; 0.1</td>
<td>6.8</td>
<td>MTC in remission</td>
</tr>
<tr>
<td>BB</td>
<td>46</td>
<td>female</td>
<td>&lt; 0.1</td>
<td>&lt; 5</td>
<td>MTC in remission</td>
</tr>
<tr>
<td>BM</td>
<td>62</td>
<td>female</td>
<td>&lt; 0.1</td>
<td>11</td>
<td>MTC in remission</td>
</tr>
<tr>
<td>KJ</td>
<td>63</td>
<td>male</td>
<td>&lt; 0.1</td>
<td>&lt; 5</td>
<td>MTC in remission</td>
</tr>
<tr>
<td>KA</td>
<td>36</td>
<td>female</td>
<td>0.15</td>
<td>9.62</td>
<td>MTC in remission</td>
</tr>
<tr>
<td>AW</td>
<td>59</td>
<td>male</td>
<td>0.16</td>
<td>40.6</td>
<td>MTC in remission</td>
</tr>
<tr>
<td>NR</td>
<td>37</td>
<td>female</td>
<td>&lt; 0.1</td>
<td>&lt; 5</td>
<td>MTC in remission</td>
</tr>
<tr>
<td>SM</td>
<td>56</td>
<td>female</td>
<td>&lt; 0.1</td>
<td>&lt; 5</td>
<td>MTC in remission</td>
</tr>
<tr>
<td>WJ</td>
<td>57</td>
<td>female</td>
<td>0.27</td>
<td>21.9</td>
<td>MTC in remission</td>
</tr>
<tr>
<td>KK</td>
<td>69</td>
<td>female</td>
<td>&lt; 0.1</td>
<td>&lt; 5</td>
<td>MTC in remission</td>
</tr>
</tbody>
</table>
Discussion

Although the prevalence of hypercalcitoninemia unrelated to MTC varies from 0.3% to 4.5% [19, 20], routine measurement of serum calcitonin level has been advocated by some authors as a possible marker of MTC among patients with nodular thyroid diseases [21, 22]. The assay’s high cost and lack of specificity [23] has dissuaded US health authorities from adopting systematic testing [23].

After surgery, patients with MTC were followed up with CT measurements. A strong correlation be-
Fig. 1. Comparisons of CT levels in the studied groups
Ryc. 1. Porównanie stężenia CT w grupach

Fig. 2. Comparisons of PCT levels in the studied groups
Ryc. 2. Porównanie stężenia PCT w grupach
between clinical stage and CT level was observed in MTC patients. However, a minimal to moderate increase of serum calcitonin level has frequently been observed in diseases other than MTC [7, 8]. Additionally, as was mentioned earlier, measuring the CT level has some laboratory inadequacies. Still, its advantages include: a constant and predictable half-life, absence of isoforms, excellent in-vitro stability and analytical consistency between assays [24]. For these reasons, there is a need to find an alternative for CT measurement in MTC. Perhaps PCT could be a better marker than CT. PCT assays are commonly used in many laboratories which do not perform CT estimations. Physiologically, PCT level is low. It is increased in bacterial and fungal infections and is considered an inflammatory marker. Moreover, it has recently been shown that PCT is a valuable tool to guide antibiotic treatment in patients with serious bacterial infections [25, 26]. Some studies suggest that a PCT-based algorithm significantly shortens the length of antibiotic therapy without affecting the treatment and outcome [25, 26]. Beyond shortening the time of antibiotic treatment, it also has a favorable effect on the time of hospitalization in the intensive care department [27].

In this study, the authors would like to assess PCT utility as a potential marker for MTC. To do this, they measured CT and PCT levels in four groups of patients.

PCT and CT levels were higher in all active MTC patients. In the re-operated patients from this group, the levels of both markers decreased post-operatively. The statistical analysis shows very strong and significant correlations between CT and PCT values in patients with active MTC. The results are similar to those obtained in other studies [27, 28].

In the group of patients in remission, both markers were not increased in 15 patients, 3 patients had both markers slightly elevated, while 5 only showed increased CT levels.

Also in this group of patients, correlations between CT and PCT values were very strong and significant. This suggests that PCT could be used as a marker in the follow-up of MTC patients.

Eleven patients with histopathologically confirmed NTNG were included in the study as a control group. PCT and CT levels in these patients were not elevated, except in one patient (DE), before routine thyroidectomy, in whom slightly elevated PCT and CT levels were observed. The fact that the PCT level is not increased in NTNG
patients and that it is increased in all active MTC patients is very hopeful. It allows authors to use PCT measurement as a screening test for MTC. Additionally, PCT measurement is cheaper and more available in many hospital laboratories.

PCT and CT levels were also checked in 30 patients with serious bacterial infections or sepsis. Only two patients, who had normal PCT levels, had slightly elevated CT levels, suggesting that CT is not a specific marker for infection or sepsis.

In the statistical analysis of this group, both parameters were completely independent.

In summary, this study showed that PCT has a similar distribution of values to calcitonin in MTC. A significant correlation was observed between these two markers in MTC patients while no correlation was seen in individuals with infection. These facts and PCT’s analytical advantages allow one to use PCT together with CT for the evaluation of MTC status. Additional prospective studies should be performed to check if PCT measurement could be an alternative to CT measurement. Present results encourage the performance of further studies.

References

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