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Calcitonin and procalcitonin measurement after cholecystokinin-2/gastrin receptor agonist stimulation in patients with advanced medullary thyroid cancer: results from the GRAN-T-MTC study

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Abstract

Introduction: Medullary thyroid cancer (MTC) is characterized by overexpression of cholecystokinin-2/gastrin receptors (CCK_2R). There are limitations of calcitonin as a tumor marker in MTC diagnosis and prognosis. Procalcitonin is gaining a role as a complementary tumor marker. This study aimed to assess the feasibility of procalcitonin measurements on top of the calcitonin measurements after CCK_2R agonist stimulation in patients with MTC.

Material and methods: The assessment was part of the GRAN-T-MTC translational study conducted through a Phase I multicenter clinical trial in patients with locally advanced and/or disseminated MTC. Patients were administered intravenously the CCK_2R agonist CP04 labelled with indium-111 ([^{111}In]In-CP04); the first four patients at a lower mass amount of 10 μg, and afterwards the whole group at a higher mass amount of 50 μg. Blood samples for calcitonin and procalcitonin measurements were obtained shortly before and 2, 5, 10, and 20 minutes after start of [^{111}In]In-CP04 administration.

Results: Sixteen patients were included in the study. After injection of the higher mass amount of [^{111}In]In-CP04, the median maximum ratio for stimulated calcitonin was 2.97 (interquartile range [IQR] 2.35) pg/mL and procalcitonin 2.01 (IQR 2.07) pg/mL. The maximum stimulated/baseline calcitonin ratio was 5.2 ± 4.0 and 4.1 ± 3.8 in the low and high mass amount groups, respectively, and the maximum stimulated/baseline procalcitonin ratio was 4.6 ± 5.1 and 2.9 ± 3.1 in the low and high mass amount groups, respectively. There was a significant linear correlation between calcitonin and procalcitonin concentrations ($p < 0.001$) at each test time point and between the maximum procalcitonin and maximum calcitonin increment ratio ($r = 0.94$, $p < 0.0001$). Mild, short-lasting side effects (transient tachycardia, flushing) were observed in one patient during the injection of low and in two patients during the injection of high mass amount of [^{111}In]In-CP04. The side effects were not related to the baseline calcitonin or procalcitonin concentrations.

Conclusion: Procalcitonin concentrations after CP04 stimulation were highly correlated with calcitonin concentrations. Unlabeled CP04, if available commercially, may be considered an alternative stimulating agent in MTC patients, even in lower mass amounts. Further studies, including healthy controls, are required to prove this concept and calculate the diagnostic thresholds. **(Endokrynol Pol 2025; 76 (3): 321–330)**

Keywords: *medullary thyroid cancer; calcitonin; procalcitonin; cholecystokinin-2/gastrin receptor agonist*

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Introduction

Medullary thyroid cancer (MTC) is a rare malignant tumor arising from neuroendocrine parafollicular cells, encompassing about 5% of all thyroid cancers [1]. 75% of MTC cases are sporadic; the remaining 25% of cases occur as part of multiple endocrine neoplasia type 2 syndrome [1]. MTC has a very peculiar clinical course with micro-metastases that are difficult to identify, often remaining stable for several years and suddenly undergoing a rapid and unrestrainable progression. MTC is responsible for approximately 13% of all thyroid cancer-related deaths [2]. While patients with a hereditary syndrome are often treated with a prophylactic thyroidectomy upon the identification of a rearranged during transfection (*RET*) germline pathogenic variant, at least 45% of the patients with sporadic MTC present with lymph node metastases and 10% with distant metastases at diagnosis [1]. The 10-year survival rate in localized disease is excellent (above 95%), but it drops significantly to 44% in patients with distant metastases present at diagnosis [2, 3].

In patients without extranodal and/or distant disease, surgery consisting of total thyroidectomy and central or modified radical neck dissection is performed with curative intent [3, 4]. The management of advanced, progressive MTC is still challenging, although therapeutic options have greatly expanded over the past decade [5]. Multidisciplinary input, including surgeons, endocrinologists, nuclear medicine physicians, medical and radiation oncologists, and other specialists, is strongly recommended to ensure optimal care for patients with advanced MTC [3]. In such cases, systemic approaches such as targeted therapy, chemotherapy, and peptide receptor radionuclide therapy (PRRT) are employed [1, 6]. The experience with PRRT, namely [^{90}Y]Y-DOTATOC and [^{177}Lu]Lu-DOTATATE, in advanced MTC is still limited; however, an objective response is observed in 10–15% of treated patients [6]. Since the expression of somatostatin type 2 receptors, the target receptors for somatostatin agonist treatment, in MTC is relatively low, there is an obvious need to develop alternative theranostic agents [7].

Therefore, with the overexpression of cholecystokinin-2 receptors (CCK_2R)/gastrin receptors on the membrane of MTC cells (in more than 90% of cases), novel radiopharmaceuticals with a high affinity to these receptors represent a potentially effective tool for molecular diagnostics and therapy in MTC patients [8, 9]. Several gastrin/CCK radioligands have been developed in the past decades as potential candidates [7]. In the first proof-of-principle study using radioiodine-labelled gastrin in a patient with a metastatic MTC, good receptor targeting was observed in the tumor lesions and body organs, including the stomach and gallbladder [10]. Among several other gastrin radioligands, CP04 was thereafter developed into a kit for the preparation of the [^{111}In]In/[^{177}Lu]Lu-CP04 theranostic pair [11, 12]. Recently published results of a Phase I study in patients with advanced/metastatic MTC showed that [^{111}In]In-CP04 is a safe and effective radiopharmaceutical with superior diagnostic performance over available conventional imaging [13]. Further comparative studies of the diagnostic performance of [^{111}In]In-CP04 in patients with confirmed or suspected MTC and clinical trials investigating the safety and efficacy of targeted radionuclide therapy with [^{177}Lu]Lu/[^{161}Tb]Tb-CP04 in patients with advanced MTC are expected in the future [14].

In the context of MTC, calcitonin is the most sensitive marker for diagnosis and follow-up [15]. However, calcitonin measurement is burdened by a series of pre-analytic, analytic, and post-analytic issues [16]. Stimulation tests with calcium or pentagastrin have been used to improve calcitonin specificity, but pentagastrin is not available anymore in many countries [5, 17, 18]. Recently, a significant body of evidence has suggested procalcitonin as an alternative tumor marker in MTC diagnosis, due to its good correlation with calcitonin [19, 20]. The data regarding procalcitonin concentrations in the setting of pentagastrin or calcium provocative tests in MTC patients had been evaluated only in a few studies [16, 21, 22].

The aim of the following analysis was to assess the feasibility of procalcitonin measurements in addition to calcitonin measurements after CCK_2R agonist stimulation in patients with advanced MTC.

Material and methods

The assessment was part of the translational study GRAN-T-MTC in the innovative field of targeted radionuclide therapy in advanced medullary thyroid cancer. The trial was conducted in the framework of the ERA-NET on Translational Cancer Research (TRANSCAN) First Joint Transnational Call for Proposals 2011 (JTC 2011) on: *Validation of biomarkers for personalized cancer medicine*. The clinical trial was conducted in accordance with international standards and with the principles of the ICH Harmonized Tripartite Guidelines for Good Clinical Practice, with applicable local regulations (including European Directive 2001/20/EC and US Code of Federal Regulations Title 21), and with the ethical principles laid down in the Declaration of Helsinki. The trial was registered at www.clinicaltrials.gov (ID: NCT03246659) and was granted an EudraCT number: 2015000 80538. The project was approved by the ethics committees in all centers participating in the clinical part of the project: the Jagiellonian University Bioethics Committee (Poland, No. 122.61201.4.2015), the National Medical Ethics Committee (Slovenia, KME 150/06/12), the Innsbruck Medical University Ethics Committee (Austria, AN2015-0229 353/2.6 354/2.1), the Azienda Ospedaliero-Universitaria Pisana Ethics Committee (Italy, Prot. No. 36449), and the Erasmus MC Ethics Committee (The Netherlands, NL55280.078.16, v04). Within the administrative/legal process,

a cooperation agreement between partners involved in the trial was signed. The Investigator's Brochure (IB) and the Investigational Medicinal Product Dossier (IMPD) were submitted and approved by the national authorities of research partners participating in the clinical trial. The design and the main outcomes of the trial are described in detail elsewhere [13].

Study population

The subjects enrolled were patients with locally advanced and/or disseminated MTC. Inclusion and exclusion criteria have been previously described [13]. Each participant provided a signed informed consent before any trial procedures were applied.

Study intervention

The patients' medical history, MTC-related signs and symptoms, physical examination, 12-lead ECG, and laboratory testing (biochemistry, complete blood count) were obtained and recorded during the screening visit. Each enrolled patient was administered intravenously a CCK_2R agonist [^{111}In]In-CP04 ([^{111}In]In-DOTA-(DGlu)6-Ala-Tyr-Gly-Trp-Met-Asp-Phe-NH2) labelled with 200 ± 10% MBq of ^{111}In. The details of [^{111}In]In-CP04 preparation and labelling have been described previously [12, 23]. The injections were performed in the morning after overnight fasting at two-week intervals. Before each injection, vital signs and a 12-lead ECG were recorded.

The first four enrolled patients (MTC01–04) had a low mass amount of 10 μg of [^{111}In]In-CP04 administered as a safety measure and a high mass amount of 50 μg of [^{111}In]In-CP04 after two weeks. The remaining trial participants were injected with a high mass amount [^{111}In]In-CP04. The time of [^{111}In]In-CP04 injection was 5 and 30 minutes for low and high mass amounts, respectively. Patients were closely monitored during and up to 48 hours post-injection. Gelofusine (B. Braun, Melsungen, Germany) was administered as an infusion of 1 mL/kg body weight over 10 min followed by a 3-h infusion of 0.02 mL/kg/min after the injection of [^{111}In]In-CP04.

In each subject, blood samples for calcitonin and procalcitonin measurements were collected shortly before [^{111}In]In-CP04 injection, as well as 2, 5, 10, and 20 minutes after the start of [^{111}In]In-CP04 administration.

The measurements were performed at the Central Laboratory participating in an external international quality assessment scheme RIQAS (RANDOX Laboratories Ltd., Crumlin, UK). The enzyme-linked immunosorbent assay (ELISA) with the Calcitonin EIA-3648 and Procalcitonin (Human) EIA-5291reagent kits (DRG®Instruments GmbH, Germany) and ELx808 spectrophotometer (BioTek R Instruments, Inc., Winooski, Vermont, United States) was used. The obtained blood samples were centrifuged (15 min, 1000 g, 18°C), and the serum after separation from the clot was aliquoted and stored at −80°C locally at each center. Serum samples were sent frozen in dry ice to the Central Laboratory. For each shipment, a temperature data logger was used to monitor and record temperature values during the whole period. The calcitonin and procalcitonin increment ratios (stimulated calcitonin concentration to baseline calcitonin concentration; stimulated procalcitonin concentration to baseline procalcitonin concentration) were calculated for each blood collection time point.

Statistical analysis

The variables were analyzed with Statistica v.13 software (TIBCO, Santa Clara, CA, USA).

Continuous data, encompassing calcitonin and procalcitonin concentrations at different time points after start of stimulation (0 minutes, 2 minutes, 5 minutes, 10 minutes, 20 minutes) and their ratios, were analyzed by linear regression. Mann-Whitney statistics were applied for the comparison of continuous values between two groups. $p < 0.05$ was considered statistically significant.

Results

Study participants' characteristics

Sixteen MTC patients, aged 30 to 66 years, were enrolled in the trial (Eastern Cooperative Oncology Group performance status at inclusion ≤ 2). None of the enrolled patients withdrew their consent during or after the trial completion.

The clinical characteristics of the study group are given in Table 1. Laboratory test results at screening are presented in Table 2. No clinical symptoms of an active infection were present at evaluation.

Calcitonin and procalcitonin concentrations during stimulation

Calcitonin and procalcitonin baseline, and calcitonin increase ratios according to the administered mass amount of [^{111}In]In-CP04, are presented in Table 3.

Median calcitonin and procalcitonin increment ratios for each time point in the high mass amount of [^{111}In]In-CP04 group are depicted in Figure 1. There was no significant difference in calcitonin and procalcitonin maximum increment ratios in patients MTC01–MTC04 when injected with low *vs*. high mass amounts of [^{111}In]In-CP04 (Fig. 2A and B, respectively).

There was a significant linear correlation between calcitonin and procalcitonin concentrations at each test time point after the injection of a high mass amount of [^{111}In]In-CP04 in MTC01–16 (Fig. 3A–E).

There was a significant correlation between the maximum procalcitonin and maximum calcitonin increment ratios after injection of a high mass amount of [^{111}In]In-CP04 in MTC01–16 (Fig. 4).

There was no significant difference in baseline calcitonin (Fig. 5A) and procalcitonin (Fig. 5B) concentrations depending on the similarity of time of maximal increase, as well as maximum calcitonin increment ratio (Fig. 5C) and procalcitonin increment ratio (Fig. 5D) depending on the similarity of time of maximum increment similarity, respectively.

The maximum calcitonin increment ratio and maximum procalcitonin increment ratio did not correlate with the baseline calcitonin and procalcitonin concentrations (Fig. 6A–B).

Side effects

Mild, short-lasting side effects (transient tachycardia and flushing) were observed in two patients during the injections of either low (MTC01, with previous history of paroxysmal tachycardia) or high (MTC-01, MTC-13) mass amount of [^{111}In]In-CP04. The side effects were not related to the baseline calcitonin or procalcitonin concentrations nor their maximum increment, as they were not observed in subjects with

Table 1. *Clinical characteristics of the patients included in the study*

Patient	Gender (M/F)	Age [years]	Age at diagnosis [years]	Hereditary *vs.* sporadic MTC	pTNM at diagnosis	TNM at inclusion	Number of MTC-related surgeries	Other significant clinical data
MTC-01	F	55	32	MEN2B	pT3N1M0	TxN1M0	4	Pheochromocytoma (metachronous bilateral adrenalectomy) Paroxysmal tachycardia
MTC-02	F	58	43	MEN2A	pT3N1M0	TxN1M0	1	Pheochromocytoma (metachronous bilateral adrenalectomy)
MTC-03	F	30	11	MEN2B	Preinvasive (diagnosed based on genetic screening)	TxN1M0	1	Pheochromocytoma (synchronous bilateral adrenalectomy)
MTC-04	M	56	42	Sporadic	pT3N1M0	Unknown (enrolled based on elevated calcitonin concentrations)	4	
MTC-05	F	63	54	Unknown	pT2N1M0	Unknown (enrolled based on elevated calcitonin concentrations)	4	
MTC-06	F	43	42	Sporadic	T4N2M1	TxN2M1	2	Type 2 diabetes Microcytic anemia
MTC-07	F	65	57	Sporadic	T1bN0M0	Unknown (enrolled based on elevated calcitonin concentrations)	1	Arterial hypertension Hyperlipidemia
MTC-08	F	35	32	Sporadic	T1bN1BMx	TxN1M0	1	Hashimoto thyroiditis
MTC-09	M	66	63	Sporadic	T3N2M0	Unknown (enrolled based on elevated calcitonin concentrations)	1	Spastic lower limb palsy Pleomorphic adenoma
MTC-10	M	49	35	Sporadic	T4N2Mx	T4N2M1	2 (2x 2003)	Postsurgical left shoulder plexus palsy; jaundice due to liver metastases; ectopic ACTH secretion; urolithiasis; radiotherapy
MTC-11	F	32	21	Sporadic	No data	Unknown (enrolled based on elevated calcitonin concentrations)	3 (2007, 2017, 2018)	–
MTC-12	F	56	33	Sporadic	TxN1bM0	TxN1bM1	1	–
MTC-13	M	50	31	Sporadic	TxN1M1	TxN1bM1	4	Liver metastases – alcohol ablation procedure (twice)
MTC-14	F	44	24	MEN2A/FMTC	TXN1M	TxN1bM1	3	Radiofrequency liver metastases ablation
MTC-15	F	57	51	Sporadic	T1bN1M0	Unknown (enrolled based on elevated calcitonin concentrations)	2	Nephrolithiasis
MTC-16	M	42	24	MEN2A	T1aN1M0	Unknown (enrolled based on elevated calcitonin concentrations)	5	

Table 2. *Baseline laboratory tests results ([14], modified)*

Blood test	Units (SI)	Median	IQR	Reference range
Hemoglobin	g/L	145	25	110–170
Hematocrit	L/L	0.44	0.08	0.35–0.5
White blood cell count	10^9/L	6.37	2.85	3–10
Platelet count	10^9/L	260	109	125–400
Thyrotropin	mIU/L	0.61	2.85	0.2–4.78
Free triiodothyronine	mol/L	4.34	1.36	3.1–6.8
Free thyroxine	pmol/L	20.2	5.1	10.3–25
Sodium	mmol/L	142	2	136–145
Potassium	mmol/L	4.24	0.31	3.5–5.5
Calcium	mmol/L	2.26	0.1	2.1–2.65
Creatinine	μmol/L	63	10	55–115
Albumin	g/L	44	3.5	32–55
Bilirubin	μmol/L	8.57	2.4	< 21
Aspartate aminotransferase	nkatal/L	346.7	110.1	< 583
Alanine aminotransferase	nkatal/L	350	267	< 750
Glomerular filtration rate	mL/min/1.73 m^2	90	30	> 60
Calcitonin	pg/mL	1520.5	26882	< 13 (F); < 30 (M)
Procalcitonin	pg/mL	194.7	204.57	< 50
Carcinoembryonic antigen	ng/mL	23.1	292.25	< 5.2

IQR — interquartile range

Table 3. *Calcitonin and procalcitonin baseline concentrations, and calcitonin and procalcitonin increase ratios according to the administered mass amounts of cholecystokinin-2/gastrin receptors agonist CP04 labelled with indium-111 [(^{111}In)In-CP04]*

Parameter	Study group	Mean (SD)	Median (IQR)
Baseline calcitonin [pg/mL]	Low mass amount of [^{111}In]In-CP04 (MTC01–04)	621.5 (366.6)	478.2 (447.2)
	High mass amount of [^{111}In]In-CP04 (MTC01–016)	25348.1 (55248.9)	1808.2 (22402.8)
Maximum increment of calcitonin (max. stimulated/baseline calcitonin ratio)	Low mass amount of [^{111}In]In-CP04 (MTC01–04)	5.2 (4.0)	4.1 (5.3)
	High mass amount of [^{111}In]In-CP04 (MTC01–016)	4.1 (3.8)	3.0 (2.5)
Baseline procalcitonin [pg/mL]	Low mass amount of [^{111}In]In-CP04 (MTC01–04)	3.9 (3.3)	3.2 (5.1)
	High mass amount of [^{111}In]In-CP04 (MTC01–016)	219.1 (454.9)	9.0 (157.6)
Maximum increment of procalcitonin (max stimulated/baseline procalcitonin ratio)	Low mass amount of [^{111}In]In-CP04 (MTC01–04)	4.6 (5.1)	2.3 (6.1)
	High mass amount of [^{111}In]In-CP04 (MTC01–016)	2.9 (3.1)	2.1 (1.9)

SD — standard deviation; IQR — interquartile range

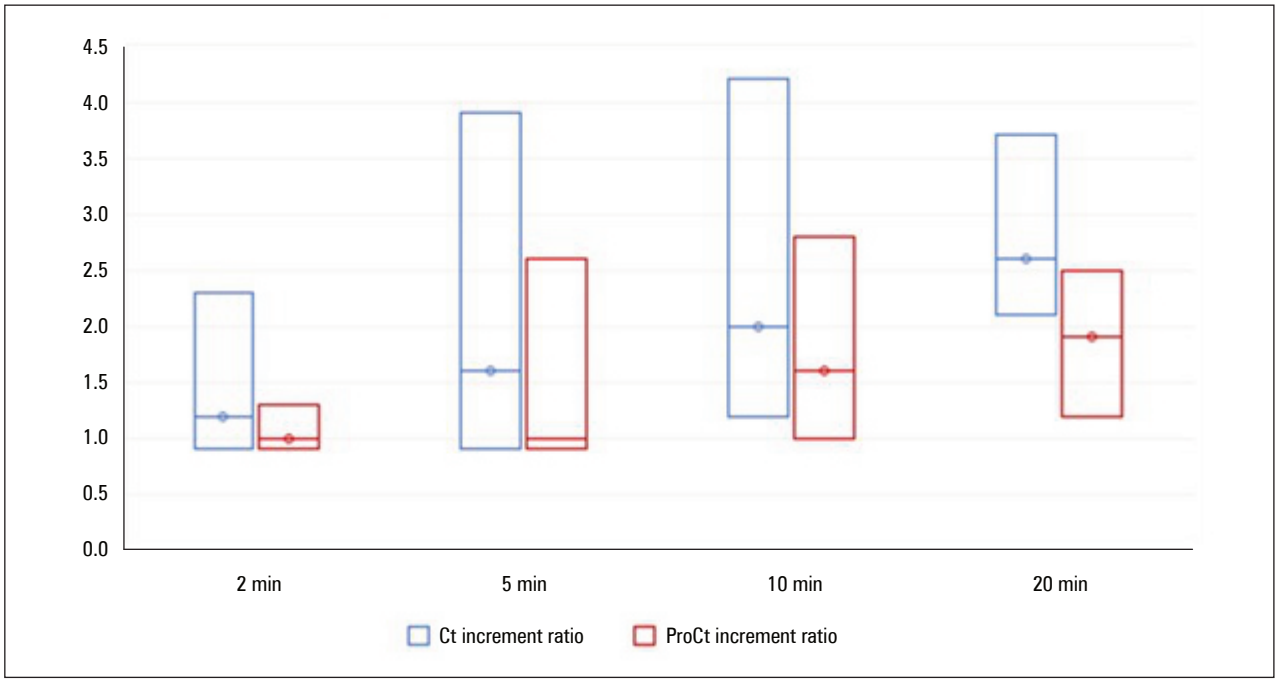

Figure 1. *Calcitonin (Ct) and procalcitonin (ProCt) increment ratios (median, interquartile range) according to the stimulation time points in the high mass amount of cholecystokinin-2/gastrin receptors agonist CP04 labelled with indium-111 [(111In)In-CP04] group (MTC01–16)*

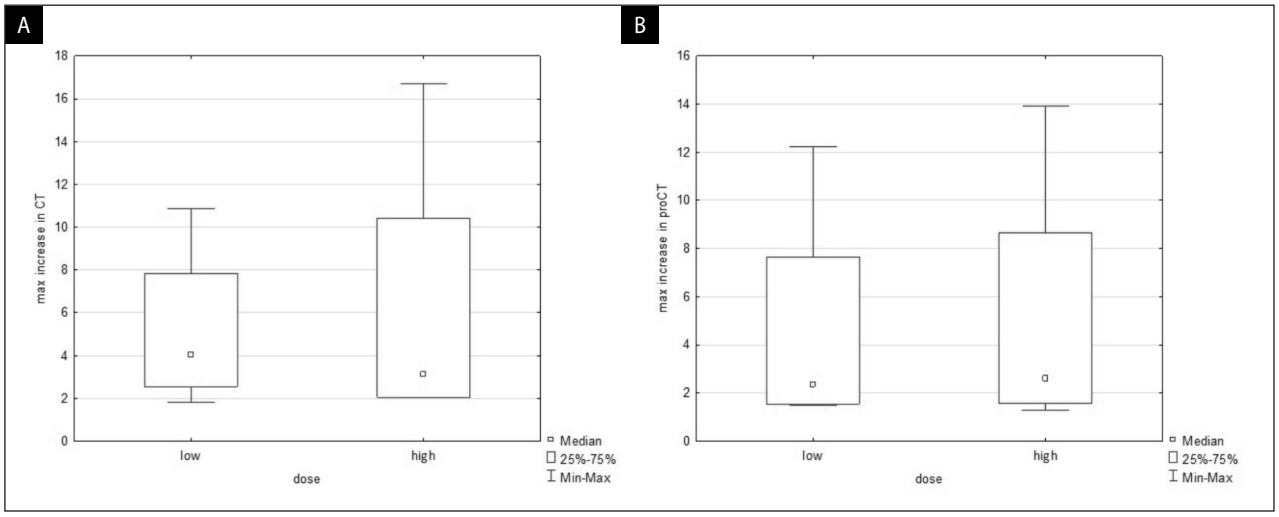

Figure 2. A. *The calcitonin maximum increment ratio in patients MTC01–MTC04, when injected with low vs. high mass amount of cholecystokinin-2/gastrin receptors agonist CP04 labelled with indium-111 [(111In)In-CP04], p = 1.0000;* **B.** *The procalcitonin maximum increment ratio in patients MTC01–MTC04, when injected with low vs. high mass amount of [111In]In-CP04, p = 0.8852*

the highest concentrations of stimulated calcitonin and procalcitonin (MTC-06, MTC-10, and MTC-12).

Discussion

Recently, the results of a phase I multicenter clinical trial of a CCK2R-seeking ligand [111In]In-CP04 in patients with advanced/disseminated MTC were published [13]. To further explore the possible clinical utility of CP04 as part of the abovementioned study, we report the results of calcitonin and procalcitonin evaluation before and after CCK_2R stimulation with [111In]In-CP04 in the same group of patients [13].

Calcitonin plays a pivotal role in MTC diagnosis and management [4, 15]. However, establishing normal intervals for calcitonin in healthy individuals and upper reference limits for men and women is challenging because commercially available assays are

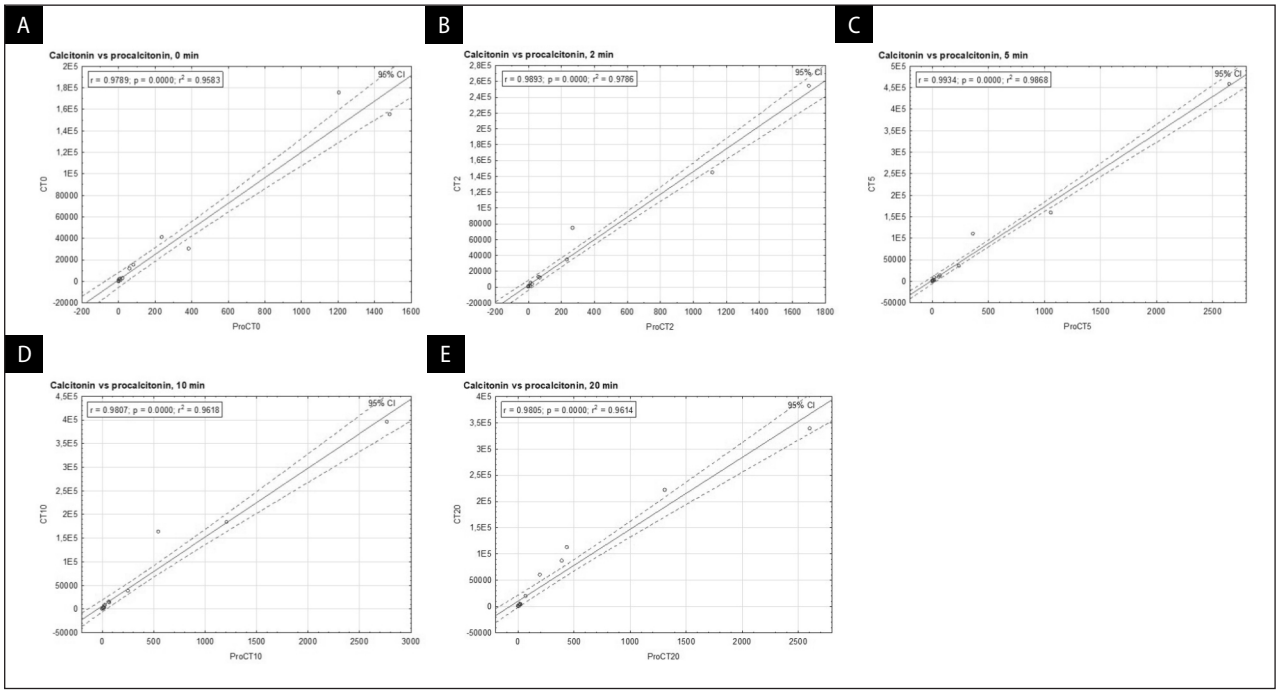

Figure 3. *The correlation between calcitonin and procalcitonin concentrations at each test time point after injection of a high mass amount of cholecystokinin-2/gastrin receptors agonist CP04 labelled with indium-111 [(111In)In-CP04].* **A.** *Before injection;* **B.** *At 2 minutes after injection;* **C.** *At 5 minutes after injection;* **D.** *At 10 minutes after injection;* **E.** *At 20 minutes after injection*

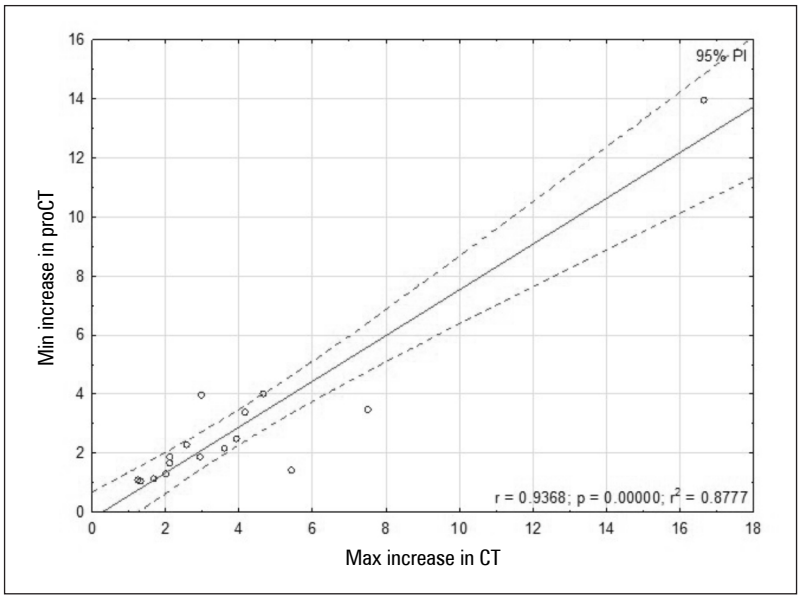

Figure 4. *The correlation between the procalcitonin and calcitonin increment ratio after injection of a high mass amount of cholecystokinin-2/gastrin receptors agonist CP04 labelled with indium-111 [(111In)In-CP04] in MTC01–16*

not comparable. Because different antibodies against calcitonin are used, some assays may also recognize calcitonin precursors. In addition, the number of C cells and thyroid volume are different in men and women [15]. Many physiological and pathological conditions, as well as commonly used medications (i.e., proton pump inhibitors, β-blockers, glucagon), may increase calcitonin concentrations. Other concerns are related to pulsatile calcitonin secretion, the influence of meals, and the instability of calcitonin at room temperature [16]. Therefore, a relatively frequent finding of basal calcitonin concentrations slightly higher than the normal range in patients with thyroid nodular disease may increase the risk of incorrect clinical decisions, including

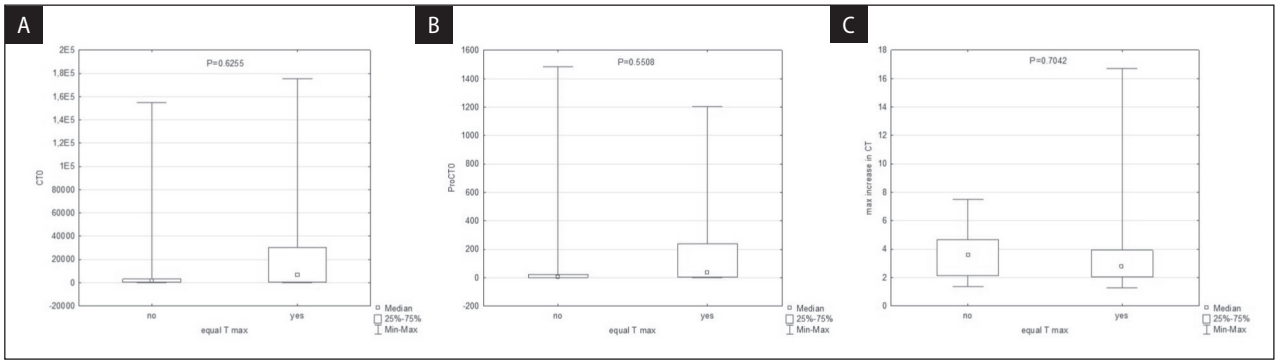

Figure 5. A. *Baseline calcitonin concentrations depending on the time of maximal increment similarity;* **B.** *Baseline procalcitonin concentrations depending on the time of maximum increment similarity;* **C.** *Calcitonin increment ratio depending on the time of maximum increment similarity;* **D.** *Maximum procalcitonin increment ratio depending on the time of maximum increase similarity*

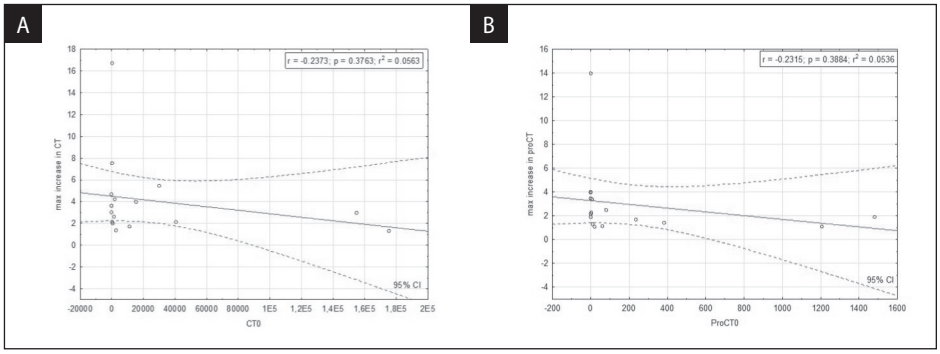

Figure 6. A. *Baseline calcitonin in correlation to maximum calcitonin increment ratio;* **B.** *Baseline procalcitonin in correlation to maximum procalcitonin increment ratio*

unnecessary thyroid biopsies and thyroidectomies [4, 15, 24].

Several approaches to overcome calcitonin limitations in MTC diagnosis and prognosis were used [5, 15, 16]. The finding of moderately high calcitonin concentrations may require a confirmatory stimulation test [4, 21, 25], based on the overexpression of some receptors found in MTCs.

Gastrin and cholecystokinin (CCK) comprise a family of structurally related peptides that exert several shared physiological actions in the gut and the nervous system via CCKR receptors located on the cell membrane of target cells [7]. CCK_2R is of particular relevance in oncology owing to its frequent and high-density expression in some human cancers, with MTC representing the most frequently CCK_2R-expressing tumor (92%) [7, 26]. Pentagastrin is a synthetic pentapeptide gastrin analog that binds to the extracellular domain of the CCK_2R and induces the release of intracellular calcitonin in patients with MTC [26]. The pentagastrin stimulation test has been used for several decades to improve the sensitivity of calcitonin as a tumor marker for MTC, but it is now omitted due to its unavailability [15, 16, 25]. Side effects, such as gastric pain, nausea, and throat tightness (usually mild-to-moderate), were recorded in approximately half of the subjects who underwent the Pentagastrin® stimulation test. No life-threatening side effects were noted [27]. Unlabeled CP04, if commercially available, might be offered as an alternative to pentagastrin, as this peptide induces an increase in calcitonin concentrations in MTC patients, as was demonstrated in our study and seems to produce side effects less frequently.

Due to pentagastrin unavailability, a calcium stimulation test has been used [16, 28]. A recent study confirmed that although basal calcitonin has shown high accuracy, some cases were diagnosed only upon stimulation [5]. When combining basal calcitonin, below or above the cut-offs, with calcium-stimulated Ct above the cut-offs, all the MTC cases were correctly identified. Besides the absolute calcitonin values, the rate of calcitonin increase should also be considered [25]. Furthermore, in cases where stimulated calcitonin was less than 2 times basal calcitonin, the measurement of the basal and stimulated procalcitonin has been proposed as an emerging concept for this clinical scenario

[21, 28]. Side effects of a calcium stimulation test are less common than during pentagastrin stimulation (approximately 40% of subjects complained of sudden warmth, 1/6 of gastric pain or throat tightness). However, one case of a life-threatening side effect (asystole) during calcium stimulation test was described, which makes the hemodynamic monitoring during the procedure essential [27].

Procalcitonin is a calcitonin precursor protein present at low serum concentrations in healthy individuals, and its concentration increases during systemic inflammation, infection, and sepsis [15]. Procalcitonin is a very stable protein, and samples for it do not require refrigeration or freezing, making it easier to handle in the pre-analytical phase [29]. In recent years, procalcitonin was suggested to be an alternative tumor marker for MTC due to its higher sensitivity and specificity, which can be used for both diagnosis and monitoring of MTC [15, 20]. Commercial procalcitonin assays give highly comparable results because they are the intellectual property of a single company [16]. Procalcitonin has also been shown to be a good marker for detecting false hypercalcitoninemia caused by heterophilic antibodies [30]. However, in a recent study, procalcitonin was undetectable in 3/14 MTC patients in the minimal residual disease group, raising some questions about its sensitivity [31]. Results of procalcitonin evaluation after calcium or pentagastrin stimulation have been reported only by a few studies [21, 22, 32]. Moreover, at present, procalcitonin measurement is not included in the guidelines for MTC management [3, 4]. In this study, we have shown that the mean maximum increment rate of calcitonin was 4 times and procalcitonin 2.9 times above the baseline level after CCK_2R stimulation with CP04, and that there is a significant linear correlation between calcitonin and procalcitonin levels, which further strengthens their role as complementary tumor markers in MTC.

With injection of [^{111}In]In-CP04, side effects following the ligand-induced CCK_2R activation should also be considered [7]. With calcium or pentagastrin stimulation tests, adverse reactions were reported, mainly as a feeling of warmth, nausea, flushing, headache, paresthesia in the extremities or lips, abdominal cramping, and urinary urgency [33, 34]. In our study, mild, short-lasting side effects (transient tachycardia and flushing) were observed in two patients during the injections of either low or high mass amounts of [^{111}In]In-CP04, resembling those elicited during the pentagastrin or calcium stimulation. Notably, there was no significant difference in calcitonin and procalcitonin maximum increment ratio in the small subgroup of patients who were injected with both low and high mass amounts of [^{111}In]In-CP04.

The limitations of this study, which was part of a phase I trial, include the small number of highly pre-selected MTC patients and the lack of a control group. This limitation should be overcome with future research in a larger and more heterogeneous group of MTC patients, also including healthy controls.

Conclusion

Procalcitonin concentrations after CCK_2R stimulation with CP04 are highly correlated with calcitonin concentrations. Unlabeled CP04 (in DOTA-unconjugated form), if available commercially, may be considered as an alternative stimulating agent in MTC patients even at low mass amounts. To prove this concept, to establish the diagnostic thresholds, and to compare its diagnostic efficacy with currently available alternatives, further studies, including healthy controls, are required.

Conflict of interests

None of the authors declared any conflict of interest.

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Ethics statement

The clinical trial was conducted in accordance with the international standards and with the principles of the ICH Harmonized Tripartite Guidelines for Good Clinical Practice, with applicable local regulations (including European Directive 2001/20/EC and US Code of Federal Regulations Title 21), and with the ethical principles laid down in the Declaration of Helsinki. The trial was registered at www.clinicaltrials.gov (ID: NCT03246659) and was granted an EudraCT number: 2015 000 805 38. The project was approved by the ethics committees in all centres participating in the clinical part of the project: the Jagiellonian University Bioethics Committee (Poland, No 122.61201.4.2015), the National Medical Ethics Committee (Slovenia, KME 150/06/12), the Innsbruck Medical University Ethics Committee (Austria, AN2015-0229 353/2.6 354/2.1), the Azienda Ospedaliero-Universitaria Pisana Ethics Committee (Italy, Prot. no 36449) and the Erasmus MC Ethics Committee (The Netherlands, NL55280.078.16, v04).

Author contributions

Concept of the study: M.T.M., P.A.E., L.L., C.D., P.K., R.M., Md.J., A.H.D., Concucting the study: M.T.M., L.L., C.D., K.Z., P.K., E.P.M., I.V., A.C.F., R.M., B.S., D.F., P.G., C.R., K.S., A.H.D.; Coordinating of the study: P.A.E., A.H.D.; Data analysis: M.T.M., K.B.S., A.S., K.S.; Drafting the manuscript: M.T.M., K.B.S., A.S.; reviewing manuscript and approval: all authors except for E.P.M. and Md.J. (deceased); final approval: M.T.M., K.B.S., A.H.D.

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Conflict of interest
The authors declare no conflict of interest.

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