Neuroendocrinology Letters Volume 37 No. 7 2016
ISSN: 0172-780X; ISSN-L: 0172-780X; Electronic/Online ISSN: 2354-4716
Web of Knowledge / Web of Science: Neuroendocrinol Lett
Pub Med / Medline: Neuro Endocrinol Lett

ORIGINAL ARTICLE

Comparative assessment of calcitonin stimulation test using calcium gluconate and pentagastrin and the usefulness of procalcitonin basic and post-stimulation concentrations in the diagnosis of patients after surgery for medullary thyroid cancer

Aldona Kowalska [1], Katarzyna Lizis-Kolus [1], Danuta Gąsior-Perczak [1], Iwona Pałyga [1], Anna Słuszniak [2], Janusz Słuszniak [3], Grażyna Antczak [4], Paweł Lizis [5], Ryszard Mężyk [1], Stanisław Góźdź [6]

1 Holycross Cancer Center, Department of Endocrinology in Kielce, Poland
2 Holycross Cancer Center, Department of Tumor Markers in Kielce, Poland
3 Holycross Cancer Center, Department of Surgical Oncology in Kielce, Poland
4 Holycross Cancer Center, Department of Laboratory Diagnostics in Kielce, Poland
5 Holycross College, Department of Physiotherapy in Kielce, Poland
6 Holycross Cancer Center, Department of Chemotherapy in Kielce, Poland

Correspondence to: Dr. Aldona Kowalska MD., PhD.
Holycross Cancer Center, Department of Endocrinology
3, Artwińskiego Str., 25-734 Kielce, Poland.
TEL: +48 413674181; FAX: +48 413674281; E-MAIL: aldonako@onkol.kielce.pl

Submitted: 2016-02-24 Accepted: 2016-03-23 Published online: 2016-12-18

Key words: **medullary thyroid cancer; calcitonin; calcium gluconate; pentagastrin; procalcitonin**

Neuroendocrinol Lett 2016; **37**(7):485–490 **PMID:** 28326742 NEL370716A02

Abstract

OBJECTIVES: The aim of the study was to compare the calcitonin (CT) stimulation tests with tests of calcium gluconate (CaG) and pentagastrin (PG), their tolerance and usefulness of PCT in the patients' diagnosis with active Medullary thyroid cancer (MCT) after thyroidectomy.

METHODS: CT was marked in serum by the immunosorbent sandwich test. PCT was marked by the immunosorbent sandwich test, with the final reading of fluorenscence. PG was given intravenously at a dose of 0.5 mg/kg body weight for 10 seconds. CaG was also given by intravenous injection at a dose of 2.5 mg of elemental Ca/kg body weight at a rate of 5ml/min, for minimum 3 minutes. Blood was taken at the 0 minute, the 3 and 5 minute after getting the stimulating substances.

RESULTS: The post-stimulation CT concentration in the 3 and 5 minute of the CaG test vs PG is significantly higher compared to the baseline. The maximal stimulation of the CT is in the 3 minute, but higher concentrations occurred using the CaG.

CONCLUSION: The results of the study suggest a similar diagnostic value of the tests with CaG compared to the PG as stimulants. In the present study we noticed a trend of basic and post-stimulation concentrations of PCT to increase in the tests with PG and CaG which correspond with the elevated concentrations of CT.

Abbreviations:
MTC - medullary thyroid cancer
CT - calcitonin
CaG - calcium gluconate
PG - pentagastrin
PCT - procalcitonin (PCT)

INTRODUCTION

MTC is a malignant tumor which derives from C cells of the thyroid and represents 3–10% of all the cancers, and 0.4–1.4% of all thyroid nodules (Elisei 2008; Giovanella *et al.* 2010; Herrmann *et al.* 2010; Sippel *et al.* 2008). The preoperative diagnosis of MTC is based on FNAB, which should be evaluated by at least two experienced pathologists in order to avoid interpretational mistakes (Wojtczak *et al.* 2012; Wojtczak *et al.* 2013).

Approximately 10–15% of the MTC is recognized postoperatively. Metastases in the lymph nodes are present in 35–50%, and distant metastases are in 10–15% of patients at diagnosis (Sippel *et al.* 2008). C cells from which MTC derives, come from neural crest and are capable of secretion of CT. It is a sensitive and specific marker of MCT used in pre- and postoperative diagnosis (Elisei 2008; Giovanella *et al.* 2010; Herrmann *et al.* 2010; Sippel *et al.* 2008; Ahmed & Ball 2011; Daumerie *et al.* 2013; Elisei *et al.* 2004; Gharib *et al.* 2010; Kaczka *et al.* 2012; Vierhapper *et al.* 2005; Walter *et al.* 2010).

It is recommended to mark CT in patients with multinodular goiter for early detection of MTC or C-Cell Hyperplasia (CCH) (Elissei *et al.* 2004; Gharib *et al.* 2010; Giovanella *et al.* 2010; Herrmann *et al.* 2010; Sippel *et al.* 2008; Vierhapper *et al.* 2005). It is estimated in the basic concentration and stimulation tests. In the estimation of the CT basic concentration there are many limitations. It may be elevated in physiological conditions such as pregnancy, lactation, neonatal period and in some diseases, such as: CCH, renal failure, mastocytosis, neuroendocrine tumors, certain leukaemias, small cell lung cancer, breast, pancreas, hyperparathyroidism, autoimmune thyroid disease (Algeciras-Schimnich *et al.* 2009). Daumerie *et al.* 2013; Giovanella *et al.* 2010; Herrmann *et al.* 2010; Kaczka *et al.* 2010; Kaczka *et al.* 2012; Levy-Bohbot *et al.* 2006).

CT is characterized by a biphasic and dependent on the concentration half-term lasting: 15th and 40th minutes in physiological concentrations, and 4 and 30 hours at elevated concentrations (Algeciras-Schimnich *et al.* 2009; Kaczka *et al.* 2010). It's rapid degradation by proteases in serum may show falsely low results (Algeciras-Schimnich *et al.* 2009; Kaczka *et al.* 2010; Pomorski *et al.* 2003). In laboratory assays different immunoreactive isoforms or the presence of heterophile antibodies significantly interfere (Kaczka *et al.* 2010; Tommasi *et al.* 2001). CT falsely low concentrations may result from the hooke effect, while falsely high (RIA method) – may occur in the presence of vitamin C, urea, creatine (Kaczka *et al.* 2010; Kaczka *et al.* 2012; Leboeuf *et al.* 2006). Another problem is the standardization of CT concentrations. The mentioned difficulties in CT assays tend us to look for other markers of MTC and testing stimulus. An alternative marker may be PTC which presence in blood was confirmed at the patients with MCT (Kaczka *et al.* 2010; Kaczka *et al.* 2012; Walter *et al.* 2010). PTC has a long half-life lasting 20–24 hours, independent of the concentration and remarkably stable in serum and plasma (Algeciras-Schimnich *et al.* 2009; Kaczka *et al.* 2010; Kaczka *et al.* 2012; Meisner *et al.* 2000; Rink *et al.* 2009). PCT advantage results from the lack of isoforms that affect the false-positive results. In patients with normal serum CT, adjusted after serum dilution, it was shown that the PTC was constantly increased. It is suggested that it is particularly useful in patients with MCT in which chaotic results by serial CT assays are obtained (Kaczka *et al.* 2010). The elevated PCT may also be a consequence of sepsis and bacterial infections, which should be excluded in the diagnosis of patients with MCT (Algeciras-Schimnich *et al.* 2009; Christ-Crain *et al.* 2006). In patients after thyroidectomy due to MCT it is recommended to perform the stimulating using tests PG or CaG to detect residual disease or relapse. It was assumed that MTC suspicion is reasonable if CT concentration after stimulation is >100 pg/ml (Ahmed & Ball 2011; Gharib *et al.* 2010). Due to the lack of availability of PG in many countries, a test of the CaG is recommended (Ahmed & Ball 2011; Daumerie *et al.* 2013; Doyle *et al.* 2009; Elisei 2008; Gharib *et al.* 2010; Giovanella *et al.* 2010; Giovanella *et al.* 2013; Herrmann *et al.* 2010; Sippel *et al.* 2008). The comparative evaluation of stimulating tests in the available literature is not clear. An important aspect is also the patients' tolerance to the substances used for the stimulation (Doyle *et al.* 2009).

The aim of the study was to compare CT stimulation tests using CaG and PG, and their tolerance in the patients' subjective assessment. Another objective was to evaluate the usefulness of PCT as a marker in the post-operative follow-up of patients with MCT in basic concentrations and after stimulation tests with CaG and PG.

MATERIALS AND METHODS

Characteristics of the test group

The study involved 40 patients with MTC, including 34 women (85%) and 6 men (15%). The mean age of patients was 56.0±17.9 (20–85 years old). In the group of 34 women's mean age was 55.0±19.0 (20–85 years old), and a group of 6 men – 61.5±9.6 (51–78 years old). All patients were treated in the Department of Endocrinology of Holycross Cancer Center in 2000–2010. The patients' characteristics based on the TNM classification (AJCC/UICC 2010) is shown in Table 1.

In 11 (27.5%) patients from the study group mutations in RET proto-oncogene was discovered. All the patients were treated with locoregional thyroidectomy

and lymphadenectomy, and with neck radiotherapy to 7 (17.5%) of them. After the surgery, the L-thyroxine replacement was applied. As the biochemical criterion for active cancer we assumed the basic or post-stimulation concentration of CT at the level >100 pg/ml. The MCT remission was observed in 35 (87.5%) patients aged 60.6±4.3, in 5 (12.5%) patients aged 55.3±19.1 we observed an active malignancy. The study excluded patients with bacterial infections and sepsis, other active cancer, liver and kidney failure, hyperparathyroidism, pregnant or lactating.

Laboratory diagnostics

CT was marked in serum by the immunosorbent sandwich test performed in the Immulite 2000 analyzer xpi Siemens. The test is based on solid-phase reactions using chemiluminescence. The scope of reference standards included the concentration to 8.4 pg/ml in men and in women to 5.0 pg/ml, with a sensitivity analysis to 2 pg/ml and a measuring range up to 2000 pg/ml. The PCT levels were determined using the assay method of Vidas Brahms PCT immunosorbent assay (sandwich assay), with the final reading of fluorenscence. The measurement range is 0.05 PCT 200 pg/ml. The reference values are <0.05 ng/dl for women and <0,09 ng/dl for men. PG (Pentagastrin injection BP, Cambridge Laboratories, Wallsend, UK) was given intravenously at a dose of 0.5 mg/kg body weight for 10 seconds. CaG (10% Calcio gluconato Monico, Monico SPA, Venezia/Mestre) was also given by intravenous injection at a dose of 2.5 mg of elemental Ca/kg body weight at a rate of 5 ml/min, for a minimum of three minutes. Blood was taken at the 0, 3 and 5 minutes after administration of the stimulating substances.

Statistical Analysis

Basic statistics (mean, SD, range) were determined for the studied parameters. The Wilcoxon test was also used. The calculations were made by software MedCalc Statistical Software version 14.10.2 (MedCalc Software byba, Ostend, Belgium; http://www.medcalc.org, 2014).

The Evaluation of the test with CaG vs PG tolerance

We made the subjective evaluation of the stimulating substances tolerance on the basis of the individual assessment questionnaire prepared in the Endocrinology Clinics in the HCCC.

Tab. 1. Characteristics of the study group patients according to the TNM classification (AJCC/UICC 2010).

TNM classification	T_1	T_2	T_3	T_4	T_x	N	M
Number of patients	22	11	4	1	2	5	1 (liver)
%	55.0	27.5	10.0	2.5	5.0	12.5	2.5

RESULTS

CT– the stimulation test with CaG

The results in stimulation of the CT with CaG test are presented in Table 2. The 0 minute vs the 3 minute and 0 minute vs the 5 minute test showed the significant increasing in the concentrations of the CT (p<0.0001). The comparative analysis of the concentrations of the CT results obtained from the 3 to the 5 minutes of the test do not show the statistical significance (p=0.4551).

CT – the stimulation test with PG

The CT concentration results obtained with PG stimulation test are shown in Table 2. The results of the analysis are consistent with the results of the test with CaG.

The differences between the basic concentrations and the stimulated ones in the 3 and 5 minutes are statistically significant (p=0.0041). The stimulated CT concentration from the 3 to the 5 minutes of the test are similar and don't show any statistical significance (p=0.3399).

Comparison of the CT stimulation tests with CaG vs PG

The results of the comparative analysis of the two tests indicate that CT concentration in both of them at the 3 and 5 minutes after stimulation is significantly higher compared with the starting concentrations: CaG test from the 0 minute to the 3 minute (p<0.0001), from the 0 minute to the 5 minute (p=0.0011); PG test from the 0 minute to the 3 minute (p=0.0041), from the 0 minute to the 5 minute (p=0.0006). There were no significant differences in the CT stimulated concentrations from the 3 to the 5 minutes of the two tests (p=0.4551, p=0.3399) (Table 2, Figure 1).

In five tested patients with active cancer (2 with persistent biochemically MCT, 2 with metastases to

Tab. 2. Comparison of CT stimulation test with CaG vs PG.

Stimulation time in minutes	0		3		5	
Parameter	CaG	PG	CaG	PG	CaG	PG
Mean	14.11	15.57	166.55	143.56	151.65	134.82
(SD)	(24.43)	(26.09)	(517.68)	(442.82)	(481.75)	(404.19)
(min–max)	(2.00–98.70)	(3.47–95.60)	(3.35–2453.00)	(3.48–2321.20)	(3.63–2342,00)	(3.65–1956.44)

p-value CaG: 0 minute vs 3 minute (p<0.0001); 0 minute vs 5 minute (p=0.0011); CaG: from 3 to 5 minute (p=0.4551); PG: 0 minute vs 3 minute (p=0.0041); 0 minute vs 5 minute (p=0.0006); PG: from 3 to 5 minute (p=0.3399)

the cervical lymph nodes, 1 with liver metastasis) we obtained the hyperstymulation of the CT concentration in both assays. In two patients (MCT biochemically survived) we found the CT hyperstymulation after the stimulation by CaG, and we stated its absence in the test with PG. In one person (MCT biochemically survived) we stated the CT stimulation limit after the CaG in the 5 minute of the test and hyperstymulation in the 3 and 5 minutes after PG.

The CaG test tolerance

The subjective side effects appeared in all the patients. In 30 of them (75%) the CaG was better tolerated than the PG, in 10 of them (25%) tolerance of both tests was the same. The oftenest side effects were feeling hot, metallic taste in the mouth and flush. The rarest ones were paraesthesia of the face, dizziness and weakness. The type and frequency of the side effects of the CaG and PG tests are shown in the Table 3.

Comparison of PCT stimulation tests with CaG vs PG

For the analysis we qualified 5 patients with active MCT. We evaluated the basic and post-stimulation PCT concentration in the 3 and 5 minutes after the intravenous administration of CaG and PG. As an indicator of cancer remission we adopted the correct basic PCT <0.1 ng/dl and lack of its stimulation in the tests with CaG and PG. The comparative analysis of PCT in both assays is presented in Table 4, Figure 2. In patients with active MCT, basic as well as stimulated PCT are elevated and correlate with CT. The highest stimulation of concentrations in relation to the 0 minute time occurs in the 3 minute in both tests, CaG and PG. The differences between the PCT concentrations from the 3 to the 5 minutes in both tests are not significant (p=0.3125, p=0.6250). The PCT levels in the 5 minute of the tests stimulation is slightly lower than in the 3 one, but the difference is not significant – p=0.0625 (Table 4, Figure 2).

Tab. 3. Subjective side effects of stimulation tests with CaG.

Symptom	The patients with side effects	
	n	%
Feeling hot	38	95.0
Metallic taste in the mouth	11	27.5
Flush	9	22.5
Increased sweating	6	15.0
Nausea	6	15.0
Paraesthesia of the face	4	10.0
Dizziness	1	2.5
Weakness	1	2.5

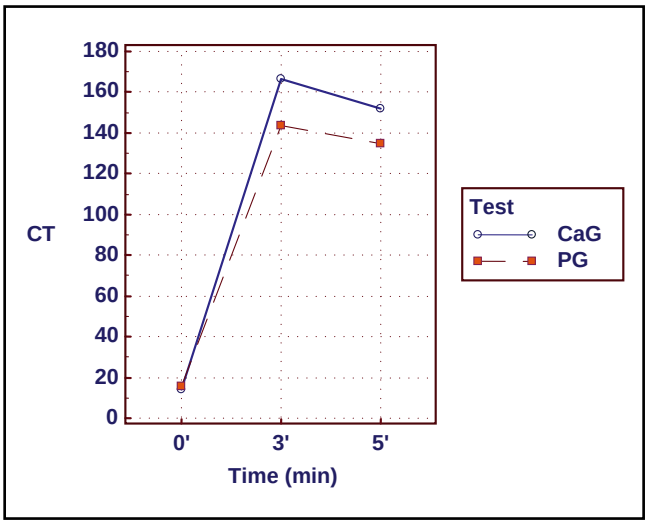

Fig. 1. Comparison of CT stimulation test with CaG vs PG.

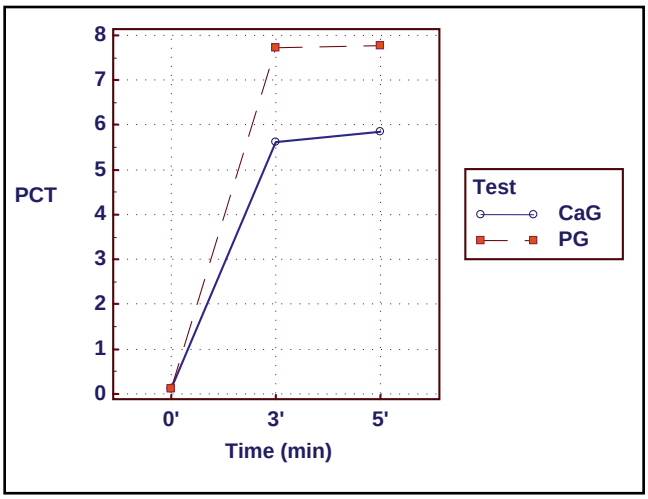

Fig. 2. Comparison of PCT stimulation tests with CaG vs PG.

Tab. 4. Comparison of PCT stimulation tests with CaG vs PG

Stimulation time in minutes	0		3		5	
Parameter	CaG	PG	CaG	PG	CaG	PG
Mean	0.10	0.12	5.62	7.73	5.85	7.78
(SD)	(0.03)	(0.03)	(7.91)	(7.33)	(8.24)	(7.08)
(min–max)	(0.09–0.17)	(0.09–0.18)	(0.72–19.57)	(1.46–15.74)	(0.67–20.36)	(1.41–15.58)

p-value CaG: 0 minute vs 3 minute (p=0.0625); 0 minute vs 5 minute (p=0.0625); CaG: from 3 to 5 minute (p=0.3125); PG: 0 minute vs 3 minute (p=0.0625); 0 minute vs 5 minute (p=0.0625); PG: from 3 to 5 minute (p=0.6250)

DISCUSSION

The retrospective study found that the CT in basic concentrations and the stimulated ones is a sensitive marker in the post-operative assessment of the patients with MCT. In the literature there are many inconsistencies concerning the basic levels of the CT concentrations allow to distinguish healthy people from the patients with CCH and MCT (Colombo *et al.* 2012; d'Herbomez *et al.* 2007; Giovanella *et al.* 2013; Mian *et al.* 2014; Rink *et al.* 2009). The aim of the study was to compare the two tests of stimulus CT – using CaG and PG. The results of the analyzes indicate that stimulation of the CT in both assays is similar, with the maximum concentrations of CT at the 3 minute of them. The abnormal post-stimulation CT concentration > 100 pg/ml was achieved in all 5 patients with active MCT, wherein after the CaG stimulation a slightly higher concentration of CT was observed, but the differences were not significant. These results are consistent with the majority of published studies, but mostly they relate to patients with multinodular goiter (Colombo *et al.* 2012; Constante *et al.* 2007; Doyle *et al.* 2009; Hampel *et al.* 2011; Milone *et al.* 2010).

Doyle *et al.* (2009) in a group of healthy adults have shown that the concentration of CT was significantly higher after 2 minutes stimulation test with CaG compared to PG assays. The similar results were obtained by Colombo *et al.* (2012), where patients with multinodular goiter obtained the CT peak after stimulation with CaG up to 2-times higher than with the PG.

In healthy individuals the similar results were obtained by Gharib *et al.* (1987) However, in patients with MCT after thyroidectomy, in the PG assay there were obtained higher CT concentrations than in the CaG one. Similar results were obtained by Hampel *et al.* (2011), where in a group of the healthy the CaG stimulator turned out to be better one and for the patients with MCT the higher CT concentrations were after the PG stimulation.

Constante *et al.* (2007) demonstrated that CT stimulation with the CaG was equally effective as with the PG in the identification of the patients with HCC and MTC. In our study we also made an analysis of PCT suitability in the diagnosis of MCT in basic concentrations and after the CaG and PG stimulation. As a correct PCT concentration we assumed PCT <0.1 ng/ml. In 5 patients with active MCT the elevated post-stimulation PCT concentrations correlated with the post-stimulation CT concentrations, CT >100 pg/ml. There were no statistically significant differences between tests with the CaG and PG. Our observation concerning the usefulness of PCT as a marker of MCT is consistent with most previous studies (Giovanella *et al.* 2010; Kaczka *et al.* 2010; Kaczka *et al.* 2012; Walter *et al.* 2010). In the available literature only the basic PCT concentration was evaluated, there were no analysis in the stimulation tests. The studies carried by Kaczka *et al.* (2010) and Kaczka *et al.* (2012) also showed a strong correlation between CT and PCT in patients with MCT. In patients with the spread of MTC the concentration of the PTC reflects active disease, as well as the CT (Kaczka *et al.* 2010).

Giovanella *et al.* (2013) showed that PCT is an even more specific marker than the CT marker for benign thyroid diseases. Walter *et al.* (2010) suggested the prognostic role of the PTC concentrations in patients with MCT. The study showed a higher diagnostic value for the CT than for the PTC in the differentiation MTC patients and the control group, but the analysis of differentiating patients with MCT and CCH indicated PTC advantage compared to CT. The authors assessed the prognostic value of the indicator PTC/CT in the differentiation of the stable and the progressive diseases (Walter *et al.* 2010). In our study we also made the evaluation of tolerance-promoting substances CaG vs PG. The results are consistent with the majority of studies available in the literature, and demonstrate that CaG test is safe and better tolerated than PG (Constante *et al.* 2007; Doyle *et al.* 2009; Hampel *et al.* 2011; Kloos *et al.* 2009; Leboulleaux *et al.* 2004; Mian *et al.* (2014).

In the conducted study, a comparative analysis of the CT stimulus tests using CaG vs PG showed no significant difference from the 3rd to the 5th minutes of the two tests. In patients with active MCT a slightly higher stimulated CT levels related to the test with CaG, but the differences were not significant. The subjective side effects of the two tests were similar and short-lived, however, the CaG test was better tolerated by most patients. Moreover, we demonstrated the PCT correlation towards the CT in basic and post-stimulation concentrations. Therefore PCT may be proposed as a marker in MCT, allowing for completion of the diagnosis.

CONCLUSIONS

In the available literature the comparative assessment of CT stimulation test with CaG and PG mainly concerns the cases of goiter and is aimed at early diagnosis of MCT. This paper presents a comparative evaluation of stimulants in the diagnosis of postoperative and follow-up of the patients with MCT. The PG difficult availability on the European market tends to look for other stimulants in the evaluation of the CT. The study's innovative side's to evaluate PCT in stimulation tests. In the literature it is suggested the role of PCT as a marker or even as a predictive factor in the diagnosis of MCT, but in basic concentrations. A small group of the patients doesn't allow to make the final conclusions. In the future it is necessary to carry out tests to assess its usefulness in the diagnosis of MCT and to carry the laboratory tests to determine the standards to distinguish patients with MCT from healthy population

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