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Clinical performance of calcitonin and procalcitonin Elecsys[®] immunoassays in patients with medullary thyroid carcinoma

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Abstract

Objectives: Medullary thyroid carcinoma (MTC) is caused by a malignant transformation in the parafollicular C-cells of the thyroid, where calcitonin (CT) is released. Nowadays, CT is the main tumor marker used in the diagnosis and follow-up of MTC patients. Nonetheless, procalcitonin (PCT) has recently been proposed as a useful complementary/alternative biomarker in MTC. Our aims were to investigate the diagnostic performance of CT and PCT and their combination in the differential diagnosis between active and inactive MTC and between MTC and non-MTC thyroid diseases, respectively.

Methods: Serum samples were collected from 16 patients with active (i.e. primary tumour before surgery or post-surgical recurrent disease) and 23 with inactive (i.e. complete remission) MTC, 125 patients with non-MTC benign thyroid disease and 62 patients with non-MTC thyroid cancers, respectively. Elecsys[®] CT and PCT measurements were simultaneously performed on the Cobas e601 platform (Roche Diagnostics, Rotkreutz, Switzerland).

Results: Both CT and PCT median values in active MTC (94 pmol/L and 1.17 ng/mL, respectively) were significantly higher compared with inactive MTC (0.28 and 0.06) and either benign (0.37 and 0.06) or malignant (0.28 and 0.06) non-MTC. Undetectable PCT was found in five non-MTC patients with false positive CT results.

Conclusions: Elecsys[®] PCT assay is a highly sensitive and specific alternative MTC marker. At the very least it appears useful in patients with positive CT results as negative PCT values securely exclude active MTC. The availability of both markers on the same automated platform facilitates reflex or reflective strategies to refine the laboratory diagnosis.

Keywords: calcitonin; immunoassay; medullary thyroid carcinoma; procalcitonin; reflex test.

Introduction

Medullary thyroid carcinoma (MTC) is a malignant tumor of the thyroid cells C cells, accounting for about 2–5% of thyroid cancers [1]. Calcitonin (CT), the main C cell secretory product, is considered a sensitive and specific marker for the diagnosis and follow-up of MTC [2]. However, CT assays suffer pre-analytic and analytic drawbacks: *i.* CT is prone to relatively rapid *in vitro* degradation by serum proteases which makes rapid processing of samples mandatory [3]; *ii.* the presence of various different immunoreactive isoforms and fragments, which can lead to inaccurate results (usually false low) as well as poor comparability of results obtained by different assays [4–7]. Carcinoembryonic antigen (CEA), a nonspecific marker, is also used for monitoring MTC patients, being a reliable predictor of disease progression [8]; however, it is an insensitive marker for early postoperative detection of MTC persistence/relapse [9]. Administering intravenous calcium or pentagastrin was largely used to improve the accuracy of CT measurement; however specificity is suboptimal and establishing clear reference guidelines for abnormal stimulated serum CT levels is a much more challenging task than establishing reference guidelines for abnormal basal CT levels. Accordingly, also considering

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additional costs and potential unpleasant side effects, most clinicians feel that provocative testing is no longer necessary [10]. Procalcitonin (PCT), the precursor of CT, circulates at low concentrations in healthy individuals; moreover, it has a concentration-independent and predictable *in vivo* half-life of 20–24 h and displays excellent *in vitro* stability in serum or plasma [11, 12]. A strong correlation between PCT and CT levels was proven in patients with MTC and multiple studies confirmed equivalent high sensitivity of CT and PCT in detecting and monitoring MTC [13–15]. On balance, the above-mentioned studies suggested that PCT, being less affected by analytical, physiological, pharmacological and pathological factors that can influence results of serum CT values, could be a new standard of care in the management of MTC [16–19]. Accordingly, Lim and colleagues demonstrated a 98–99% negative predictive value for pathologically increased CT levels [20]. Recently, a new CT immunoassay become available on automated Cobas® platforms (Roche Diagnostic, Rotkreuz, Switzerland) [21, 22] making feasible both simultaneous and sequential (reflex) measurement of CT and/or PCT on the same platform. Therefore, the present study was undertaken to compare the clinical performance of the automated Elecsys® CT and PCT assays and their combination or sequential use for the diagnosis of MTC.

Materials and methods

This study was approved by the Ente Ospedaliero Cantonale Institutional Review Board and the Canton Tessin Ethical Committee, Bellinzona (Switzerland) [references CE 3466 and BASEC 2019-00662, respectively]. The study was performed in accordance with the World Medical Association Declaration of Helsinki regarding ethical conduct of research involving human subjects. A total of 226 subjects were included:

- (1) Patients with confirmed active MTC (n=16, 5 males, aged 31–78 yr; 11 females, aged 22–75 yr), based on clinical, imaging, or histological evidence of initial, persistent or recurrent disease. Patients with active MTC included six cases of newly diagnosed disease and 10 cases of metastatic disease.
- (2) Patients with confirmed MTC, deemed disease free by clinical assessment, imaging, and serum CT determinations (inactive MTC; n=23; five males, aged 22–73 yr; 18 females, aged 19–82 yr).
- (3) Patients with untreated benign thyroid diseases, including benign thyroid nodules, hyperthyroidism, hypothyroidism and goiter (n=125; 27 males, aged 26–75 yr; 98 females, aged 18–79 yr).
- (4) Patients with untreated (i.e. preoperative sampling) non-medullary differentiated thyroid cancer (DTC) (n=62; 19 males, ages 18–84 yr; 43 females, aged 20–76 yr).

The specimens were obtained from residual samples from patients of our Centre who had undergone CT and other thyroid testing at the EOLAB Central Laboratory (Bellinzona, Switzerland) between June 1st,

2016 and May 30th, 2018. Sera were stored at –80 °C immediately after clinical testing, until testing for Elecsys® CT and PCT was performed for the present study. The final diagnosis and assessment of disease status was based on the longitudinal review of the clinical, imaging and biochemical data and any cytology/histology data available. Patients with renal failure were excluded because this may result in serum CT and PCT elevations.

Analyte measurements

For the purpose of the present study CT and PCT were measured on a fully automated analyzer Cobas e601 (Roche Diagnostics, Rotkreuz, Switzerland) that uses electrochemiluminescence (ECL) technology for immunoassay analysis (<https://diagnostics.roche.com/global/en/products/instruments/cobas-e-601.html> as last accessed on 20.09.2020). The Elecsys® Calcitonin assay is standardized against IRP WHO 89/620 international standard. Limits of blank (LoB), detection (LoD) and quantification (LoQ, with a total allowable error of $\leq 30\%$) values are 0.09 pmol/L, 0.14 pmol/L/L, 0.28 pmol/L, respectively (<https://diagnostics.roche.com/global/en/products/params/elecsys-calcitonin.html> as last accessed on 20.09.2020). As the intellectual property for commercial PCT assays is licensed out by BRAHMS® GmbH (Henningdorf, Germany) the Elecsys® BRAHMS procalcitonin assay is standardized against BRAHMS® PCT luminescent immunoassay (LIA). Analytical (AS) and functional (FS) sensitivity values are 0.02 ng/mL and 0.06 ng/mL, respectively (<https://diagnostics.roche.com/fi/en/products/params/elecsys-brahms-procalcitonin-pct.html> as last accessed on 20.09.2020). All assays were performed in strict adherence to the manufacturers' instructions.

Data analysis and statistics

For the purpose of statistical analysis, measurement results below the FS were replaced with 0.28 pmol/L and 0.06 ng/mL for CT Elecsys® and PCT Elecsys®, respectively. For all variables, differences in the distribution of the values among the four different subgroups of patients were estimated with Kruskal-Wallis test and in case of positive result a post-hoc pairwise comparison of subgroups was performed according to Conover [23]. All continuous variables were dichotomized, using receiver-operating characteristic (ROC) analysis and Youden's coefficient (index J) to identify the optimal cut-off point to discriminate between active MTC patients from subjects with complete remission MTC. Data analysis and ROC curves plotting were performed using MedCalc Statistical Software version 19.4.1 (MedCalc Software Ltd, Ostend, Belgium; 2020). For all tests a p-value <0.05 was considered statistically significant.

Results

The distribution of serum CT and PCT concentrations in patients with active MTC, inactive MTC, benign and malignant non-MTC thyroid diseases is showed in Figure 1.

Median serum concentrations of both markers were significantly higher in patients with active MTC compared

Figure 1: Distribution of serum CT and PCT in patients with active MTC, inactive MTC, non-MTC benign and non-MTC malignant thyroid diseases, respectively [squares, median values; bars, interquartile ranges (IQR)].

with other groups. In addition, higher CT levels were found in patients with non-MTC benign disease compared to both inactive MTC and non-malignant MTC, respectively (Table 1).

No overlaps of PCT values were observed between patients with active MTC compared to other groups. Accordingly, the highest value in non-MTC patients (i.e. 0.20 ng/mL) was adopted as cutoff point to maximize the specificity without detriment in sensitivity. Applying the above described cutoff levels the corresponding sensitivity, specificity, positive (LHR+) and negative (LHR-) likelihood ratio values were 100%, 100%, 210 and 0 respectively. Discrepant results were found in five patients affected by non-MTC benign disease showing true-negative PCT but false-positive CT results, respectively (Table 2).

Since gender and thyroid volume is a relevant determinant for CT levels in non-thyroidectomized patients the performance of CT in discriminating patients with active MTC from those carrying benign and malignant non-MTC diseases was also evaluated by using *i.* the 97.5th percentile of CT distribution in males (3.7 pmol/L) and

females (3.3 pmol/L) with non-MTC thyroid diseases (benign and malignant) and *ii.* the manufacturer-provided cutoff (males 2.8 pmol/L, females 1.9 pmol/L). Results are summarized in Table 3.

Discussion

This retrospective study evaluated the use of Elecsys® CT and PCT as tumor markers in patients with MTC. First of all, no statistically significant difference was found between the two analytes in discriminating active MTC from MTC in complete remission. However, when the ROC curve-optimized cutoff used for the above purpose was adopted to discriminate active MTC from patients with non-MTC thyroid disease false-positive results were observed in five patients, affected by Graves' disease ($n=1$) and multinodular goiter ($n=4$). This is not unexpected as CT levels increase in patients with enlarged thyroid glands; conversely these levels are low in patients with complete remission MTC due to previous surgery and absence of active disease. In addition gender-specific

Table 1: Comparison of calcitonin and procalcitonin levels in different patients' groups (data are expressed as median and interquartile range, IQR).

	MTC		Non-MTC		Kruskal-Wallis p-Value	Conover test p<0.05
	Active (1)	Inactive (2)	Benign (3)	Malignant (4)		
CT, pmol/L	94.10 (13.1–283)	0.28 (0.28–0.74)	0.37 (0.28–1.13)	0.28 (0.28–0.48)	<0,0001	1 vs. 2,3,4 2 vs. 1 3 vs. 1.4 4 vs. 1.3
PCT, ng/mL	1.17 (0.45–3.46)	0.06 (0.06–0.06)	0.06 (0.06–0.06)	0.06 (0.06–0.06)	<0,0001	1 vs. 2,3,4 2 vs. 1 3 vs. 1 4 vs. 1

ROC curves for CT were plotted using MTC patients with active (sensitivity) and inactive (specificity) disease and showed a 100% sensitivity and specificity [area under the curve (AUC) of 1.00 (95% C.I. 0.91–1.00), $p<0.001$]. The best cutoff value was derived from ROC curve analysis (Youden index J) and settled at 3.1 pmol/L (Figure 2).

Table 2: Patients with discordant calcitonin (cutoff 3.1 pmol/L) and procalcitonin (cutoff: 0.20 ng/mL) results.

Patient	Gender	Age	Diagnosis	CT (pmol/L)	PCT (ng/mL)
1	M	49	GD	3.70	0.06
2	M	53	MNG	4.30	0.06
3	F	53	MNG	3.40	0.06
4	F	54	MNG	4.10	0.06
5	F	74	MNG	4.30	0.08

GD, Graves' disease; MNG, multinodular goitre; CT, calcitonin; PCT, procalcitonin.

Table 3: Calcitonin: discrimination of patients with active MTC from those with non-MTC thyroid diseases using different cutoff points.

Cutoff (criteria)	Cutoff (pmol/L)	Sensitivity	Specificity	+LHR	–LHR
MTC ROC	3.10	100%	96.7%	30	0
Non-MTC 97.5th ^a	3.70	100%	96.7%	30	0
Non-MTC 97.5th ^b	3.30	100%	97.6%	42	0
MCO ^a	2.80	100%	90.0%	10	0
MCO ^b	1.90	100%	95.0%	21	0

+LHR, positive likelihood ratio; –LHR, negative likelihood ratio; MTC, medullary thyroid cancer; ROC, receiver operating characteristic curve; MTC ROC, ROC-derived cutoff obtained in MTC patients; non-MTC 97.5th, 97.5th percentile value obtained in non-MTC patients; MCO, manufacturer-provided cutoff; ^a males ^b females.

Figure 2: Receiver operating characteristic (ROC) curve for calcitonin (sensitivity: active MTC, specificity, inactive MTC).

cutoff are suggested to improve accuracy. Accordingly, gender-specific cutoff levels obtained in our patients with non-malignant thyroid disease and those provided by the

manufacturer and obtained in healthy subjects were also applied. No differences in sensitivity (100%) were observed using different cutoff levels (ranging from 2.8 to 3.7 pmol/L in males and 1.9–3.3 pmol/L in females, respectively). While manufacturer-provided cutoffs reduced specificity in both males and females those obtained in our patients with non-MTC thyroid diseases performed equally to ROC-derived one in males and slightly increased specificity and +LHR were observed in males and females as well. In summary, as the main results of our study *i.* both Elecsys® CT and PCT assays were highly sensitive and specific MTC markers; *ii.* in the few cases with false-positive CT results a negative PCT measurement safely excluded active MTC. Putting our results in current clinical setting and considering that MTC management is centered on CT measurement since decades it seems reasonable, at the very least, to adopt PCT as complementary if not even as a replacement marker in patients with thyroid nodules and positive CT screening as well as in MTC patients with unclear serial CT measurement patterns after thyroidectomy. Furthermore, the present results, again confirming the value of PCT in MTC care, should contribute to long-term follow-up studies in MTC using PCT in order to further facilitate adoption of this novel, more reliable marker in MTC care. Having both assays performed on the same automated platform greatly facilitates the adoption of reflexing or reflective testing strategies avoiding inappropriate, even invasive, procedures to our patients with attached costs.

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