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Circulating pro-gastrin releasing peptide (ProGRP) in patients with medullary thyroid carcinoma

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Abstract

Objectives: Serum calcitonin (CT) is pivotal in medullary thyroid cancer (MTC) management. Recently, progastrin releasing peptide (ProGRP) has been proposed as a candidate complementary tumor marker of MTC. As current data are sparse our study was undertaken to evaluate the distribution of ProGRP in patients with MTC and its relationship with the tumor burden. Additionally, serial measurement of CT, carcinoembryonic antigen (CEA) and ProGRP was evaluated in three patients undergoing tyrosine kinase inhibitors (TKI).

Methods: Seventy-eight, 125 and 62 sera from patients with MTC, non-medullary malignant and benign thyroid diseases were collected, respectively. ProGRP measurement was performed by Elecsys® assays on Cobas e601 platform (Roche Diagnostics).

Results: Significantly higher ProGRP levels were found in MTC compared to non-MTC patients. Among MTC patients ProGRP levels accurately discriminate patients with active from those with cured disease and, respectively, patients with loco-regional active disease from those with distant metastasis. Finally, ProGRP performed better than CT and CEA in monitoring the response to TKI therapy in three patients monitored serially.

Conclusions: Serum ProGRP is promising as a complementary tumor marker in MTC patients. Further studies will be required, mainly focused on monitoring ProGRP during TKI treatment for early detection of resistance and assessing its usefulness to avoid the observed false positive fluctuations that occur with CT and carcinoembryonic antigen.

Keywords: calcitonin; carcinoembryonic antigen; medullary thyroid carcinoma; progastrin releasing peptide (ProGRP); thyroid tumor.

Introduction

Medullary thyroid carcinoma (MTC) is a neuroendocrine tumor caused by a malignant transformation in the parafollicular C cells of the thyroid and constitutes between 5 and 10% of all thyroid carcinomas. MTC may occur either as hereditary and sporadic forms. The hereditary form, which represents 25% of MTC can be found in multiple endocrine neoplasia 2A or 2B or as part of familial MTC based on a specific germline mutation in the RET proto-oncogene (RET) [1]. The main tumor biomarker used in both diagnosis and surveillance following primary treatment of MTC is calcitonin (CT), the principal C cell secretory product [2]. Unfortunately, normal preoperative levels of CT cannot always rule-out a diagnosis of MTC [3]. In addition, CT measurement suffers pre-analytic and analytic drawbacks which makes rapid processing of samples mandatory and potentially produce inaccurate results and poor comparability between different assays [4]. In this instance, procalcitonin, a precursor of CT, has also been studied with interesting features and some advantages when compared to CT [5]. MTC is also known to produce carcinoembryonic antigen (CEA) in about 50% of cases. However, it serves well as marker for disease progression in relapsed/advanced MTCs but it is insensitive

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for either preoperative diagnosis or early postoperative assessment [6]. Several studies have demonstrated the presence of gastrin-releasing peptide (GRP), a member of the bombesin-like family, in MTC tissue suggesting its potential use as circulating biomarker in MTC patients [7, 8]. The GRP instability in blood makes difficult its measurement in clinical practice, nonetheless, it is possible to use its stable precursor, the progastrin-releasing peptide (ProGRP) [9]. Some preliminary studies suggest that ProGRP may be helpful for the diagnosis of MTC and for monitoring the response to therapy [10, 11]. However, prevalence of this malignancy is low and small series were evaluated. Therefore, the present study was undertaken to investigate the clinical performance of ProGRP in a large series of patients affected by MTC at different stages. Additionally, serial serum ProGRP measurement was obtained in three patients with advanced MTC under thyrosin kinase inhibitor(TKI) therapy.

Materials and methods

This study was approved by the Ente Ospedaliero Cantonale Institutional Review Board and the Canton Ticino Ethical Committee, Bellinzona (Switzerland) [references CE 3466 and BASEC 2019-00662, respectively]. Informed consensus was obtained from all subjects involved in the present study. The study was performed in accordance with the World Medical Association Declaration of Helsinki regarding ethical conduct of research involving human subjects. The specimens were obtained from residual samples from patients who had undergone CT testing in our centers between June 1st, 2016 and December 31st, 2019. Sera were stored at −80 °C immediately after clinical testing, until testing for ProGRP was performed for the present study. Samples were included if (i) the residual sample volume was greater than 0.5 mL, and (ii) if the sample had undergone no more than one freeze/thaw cycle. Assignment of disease status was done by an experienced thyroid specialist (L.G.) based on the longitudinal review of the clinical, imaging, and biochemical data (including serum CT levels) and any cytology/histology data available. Patients were classified as alive with no evidence of structural disease (NESD) if there was no clinical, imaging, or cytological/histological evidence of disease at the time of the blood draw and their serum CT and CEA were either undetectable or detectable but below the upper reference limits and unchanged over several visits. Patients who did not fulfill these criteria were classified as alive with evidence of structural disease (ESD) and further stratified into alive with loco-regional (LR-ESD) disease or alive with distant metastasis (MTS-ESD).

Overall, 265 sera from 207 unique patients were included as detailed hereafter:

(1) Patients with MTC and ESD after surgery (sera, n=29; pts, n=14, four males, aged 31–78 years; 10 females, aged 22–75 years). Among them 19 and 10 sera corresponded to LR-ESD and MTS-ESD, respectively.
(2) Patients with MTC and NESD after surgery (sera, n=49; pts, n=23, nine males, aged 22–73 years; 14 females, aged 19–82 years).
(3) Patients with benign thyroid diseases, including benign thyroid nodules, hyperthyroidism, hypothyroidism and goiter (sera, n=125; pts, n=125, 27 males, aged 26–75 years; 98 females, aged 18–79 years).
(4) Patients with non-medullary differentiated thyroid cancer (DTC) (sera, n=62; patients, n=45, 19 males, ages 18–84 years.; 26 females, aged 20–76 years).

Additionally, ProGRP was measured on residual specimens serially collected in patients (n=3) under TKI treatment and compared with CT and CEA results and computed tomography findings.

Progastrin releasing peptide measurement

Serum ProGRP concentrations were measured using the Elecsys® ProGRP immunoassay on a fully automated electrochemiluminescence (ECLIA) e601 Cobas® platform (Roche Diagnostics, Rotkreuz, Switzerland). The limit of detection (LoD), limit of quantification (LoQ) and measuring range are 3 pg/mL, 7 pg/mL (≤30% total error) and 3–5,000 pg/mL, respectively, as quoted by the manufacturer (https://www.cobas.roche.it/home/product/clinical-and-immunochemistry-testing/elecsys-progrp-assay.html; accessed on 18.03.2021).

Data analysis and statistics

For the purpose of statistical analysis, ProGRP results below the LoD were replaced with 7 pg/mL, respectively. Distributions of ProGRP levels are reported as median and interquartile range (IQR). Differences in the distribution of the values among 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 [12]. Serum ProGRP concentrations were dichotomized using receiver-operating characteristic (ROC) analysis and Youden's coefficient (index J) to assess the diagnostic performance and select the best ProGRP diagnostic threshold. Data analysis and ROC curves were elaborated using MedCalc® Statistical Software version 19.4.1 (MedCalc® Software Ltd, Ostend, Belgium; 2020). For all tests a $p<0.05$ was considered statistically significant.

Results

Serum ProGRP concentrations were significantly higher in patients with active MTC (median 880 pg/mL, IQR 576.5–1,271.2 pg/mL) compared with those with cured MTC (median 74.8 pg/mL, IQR 47.7–146.8 pg/mL) and non-medullary thyroid diseases (median 46.3 pg/mL, IQR 36.8–57.7 pg/mL), respectively ($p=0.0066$). Moreover, as summarized in Table 1, significantly higher ProGRP concentrations were found in patients with MTC ESD compared to those with MTC NESD, non-medullary benign and malignant thyroid diseases ($p<0.0001$), respectively. No differences were found between patients with cured MTC (NESD) and those with non-medullary thyroid disease (benign and malignant), respectively.

Table 1: Serum ProGRP levels in different patients' groups (data are expressed as median and interquartile range, IQR).

Patients	MTC (n=78)		Non-MTC (n=187)		Kruskal–Wallis	Conover test
MTC status	1. ESD (n=29)	2. NESD (n=49)	3. Benign (n=125)	4. Malignant (n=62)	p-value	p<0.05
ProGRP, pg/mL	880.0 (576.5–1,271.2)	74.8 (47.7–146.8)	44.9 (34.9–57.3)	47.2 (37.5–58.1)	<0.0001	1 vs. 2,3,4
						2 vs. 1
						3 vs. 1
						4 vs. 1

MTC, medullary thyroid cancer; ESD, evidence of structural disease; NESD, no evidence of structural disease; ProGRP, progastrin releasing peptide.

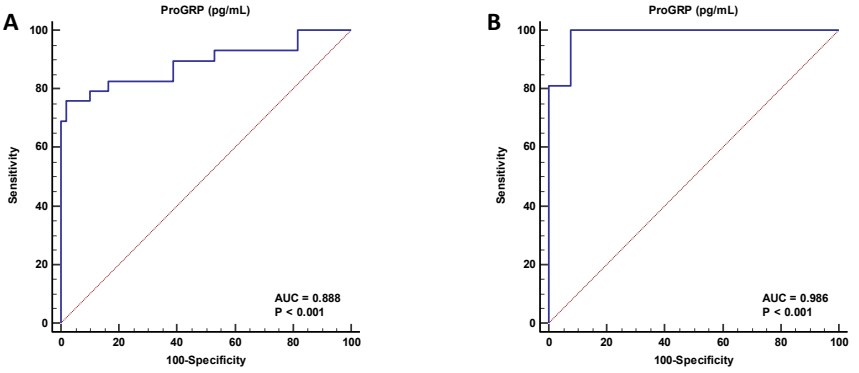

Figure 1: ROC curves plotted using MTC ESD (sensitivity) vs. MTC NESD (specificity) [AUC: 0.89 (95% CI 0.91–1.00), p<0.001] (A) and MTC ESD-LR (sensitivity) vs. MTC ESD-MTS (specificity) [AUC: 0.99 (95% CI 0.91–1.00), p<0.001] (B).

ROC curves were plotted using MTC ESD (sensitivity) vs. MTC NESD (specificity) [AUC: 0.89 (95% CI 0.91–1.00), p<0.001] (Figure 1A) and MTC ESD-LR (sensitivity) vs. MTC ESD-MTS (specificity) [AUC: 0.99 (95% CI 0.91–1.00), p<0.001] (Figure 1B), respectively. The ROC-derived cut-off levels (Youden's index J) were settled at 72.2 and 167 pg/mL, respectively. The corresponding figures of merit are summarized in Table 2.

Finally, serum ProGRP was measured on available specimens from three patients with advanced metastatic MTC treated with vandetanib (Caprelsa®, Sanofi Genzyme, USA). Serum ProGRP, CT and CEA were measured at baseline and every three months and compared with the corresponding computed tomography examination, assessed according to the RECIST 1.1 criteria (https://recist.eortc.org/recist-1-1-2/ accessed on 25.03.2021). Serum CT and CEA were measured on Immulite®2000 (Siemens Healthineers, Erlangen, Germany) and e601 Cobas® (Roche Diagnostics, Rotkreuz, Switzerland) platforms, respectively. As displayed in Figure 2 changes in serum ProGRP and CT levels matched RECIST 1.1 criteria in 10 (91%) and 8 (73%) of 11 visits, respectively. Decreasing serum CT in patients with stable disease were recorded in two observations (18%) while increasing ProGRP and CT occurred in one observation (9%) with a concurrent stable disease at imaging. In the latter case, however, increasing ProGRP and CT correctly anticipated structural relapse as recorded three months later. Finally, serum CEA levels fluctuated over time in all patients without providing additional information compared with CT and ProGRP.

Table 2: Diagnostic performance of serum ProGRP in discriminating MTC patients with different disease status.

MTC status	ROC-derived cut-off	Sensitivity	Specificity	LHR–	LHR+
ESD vs. NESD	72.2 pg/mL	75.9%	97.9%	0.25	37.2
ESD-LR vs. ESD-MTS	167 pg/mL	100%	96.3%	0	13

MTC, medullary thyroid cancer; ESD, evidence of structural disease; NESD, no evidence of structural disease;LR, loco-regional; MTS, distant metastasis.

Discussion

This retrospective study evaluated the performance of serum Elecsys® ProGRP measurement as tumor marker in patients with MTC at different stages. Significantly higher levels of ProGRP were found in patients affected by MTC compared to those with non-medullary thyroid disease and those with active compared to cured MTC, respectively. Overall, our data corroborated those reported by Parra-Robert et al. [13]. Additionally, serum ProGRP proved to accurately discriminated patients with loco-regional and distant MTC metastasis. Overall, the good correlation between serum ProGRP and the disease status (active vs.

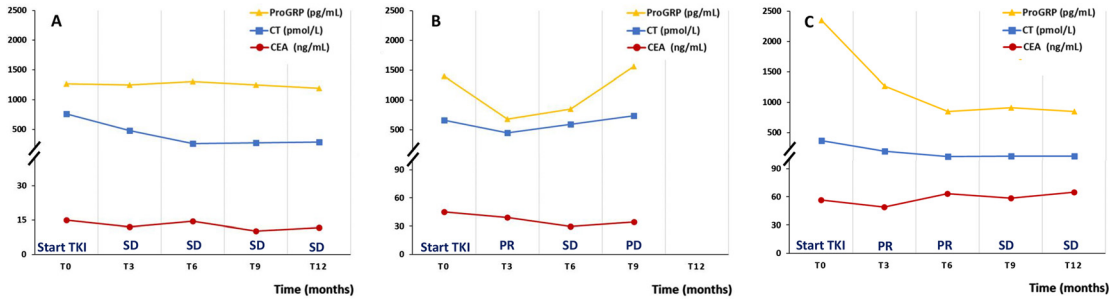

Figure 2: Serial measurement of ProGRP, CT and CEA and corresponding computed tomography-based restaging.
(A) 52 years-old female, metastatic MTC (lymph nodes, lung, bone); (B) 67 years-old male, metastatic MTC (lymph nodes, lung, liver, bone); (C) 44 years-old female, metastatic MTC (lymph nodes, liver, brain). ProGRP, progastrin releasing peptide; CT, calcitonin; CEA, carcinoembryonic antigen; TKI, thyrosin kinase inhibitor; SD, stable disease; PR, partial response; PD, progressive disease.

cured) and tumor burden make it suitable as serum marker of MTC. Notably, ProGRP assay shows relevant pre-analytical (i.e. *in vitro* stability) and analytical (i.e. lack of susceptibility to interfering isoforms or fragments, which cause false-low CT results) making unlikely aspecific fluctuations due to pre(-analytical) problems. Nevertheless, whether ProGRP is useful or not in combination with CT will need to be demonstrated by prospective studies. In facts, our patients were classified basing on CT levels and the best performance an alternative marker (i.e. ProGRP) can show is equal to that of comparator (i.e. CT). Interestingly, as the main result of our study, serial measurements of ProGRP correlated with the structural response to vandetanib better than CT and CEA, respectively. An initial decline of both markers has been previously reported in nearly all MTC patients after starting vandetanib, followed in one-third of them by transient fluctuations, including spikes above baseline, despite the absence of radiological progression [14, 15]. The cause of divergent ProGRP and CT kinetics during TKI therapy has not been elucidated yet. Vandetanib inhibits proto-oncogene RET, that is pivotal in treating MTC, but also epidermal growth factor (EGF), vascular endothelium growth factor 2 (VEGF2), and VEGF3 receptors, respectively [16]. A negative relationship between serum ProGRP levels and response to EGFR-targeting TKIs was reported in patients with EGFR-mutated non-small cell lung cancer [17]. Accordingly, vandetanib modulates the biosynthesis and secretion of ProGRP and CT via different pathways and this likely contributes to divergent trends observed during treatment.

Conclusions

Serum ProGRP is a promising MTC biomarker and, especially, shows a better relationship with structural changes during TKI therapy when compared with serum CT and CEA. Further prospective studies will be required to define more precisely in which clinical situations ProGRP measurement is useful in addition to CT and CEA. Basing on our current results it seems reasonable to consider the inclusion of ProGRP as complementary tool in MTC patients with unclear serial CT and CEA measurement patterns and/or incongruent CT and CEA levels and imaging studies.

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Competing interests: L.G. is a member of Roche Diagnostics Advisory Board and has received research grants from Roche Diagnostics and speaker honoraria from Roche Diagnostics and ThermoFisher BRAHMS. Other authors disclose no conflicts of interest.
Informed consent: Informed consent was obtained from all individuals included in this study.
Ethical approval: This study was approved by the Ente Ospedaliero Cantonale Institutional Review Board and the Canton Ticino Ethical Committee, Bellinzona (Switzerland) [references CE 3466 and BASEC 2019-00662, respectively].

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