

Review

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Evolution of thyroid cancer biomarkers: from laboratory test to patients' clinical management

<https://doi.org/10.1515/cclm-2022-1087>

Received October 27, 2022; accepted October 31, 2022;

published online November 11, 2022

Abstract: Over the past three decades, laboratory medicine has significantly evolved thanks to technological advances made possible by new materials and evidence. Clinicians' ongoing requests for powerful, rapid, and minimally invasive tests has led manufacturers to develop rapid, accurate, and sensitive tests that can increase diagnostic accuracy and improve follow-up, bringing laboratory medicine ever closer to personalized medicine. The aim of this study was to critically review the main problems of the current Tg and CT biomarkers for the diagnosis/monitoring of DTC and MTC, respectively, and to identify the advantages and challenges of using the new laboratory biomarkers in the clinical management of patients with differentiated and medullary thyroid cancer. Insufficient harmonization of Tg and CT assays and lack of interchangeability of laboratory results and cutoff values pose challenges for comparability and standardization of procedures and methods. New diagnostic and monitoring approaches such as PCT or the Tg doubling time have proven to be effective. Close collaboration between clinicians and laboratory specialists remains

essential to translate the advantages and limitations of current assays into appropriate clinical interpretation criteria. Over the years, the journal *Clinical Chemistry and Laboratory Medicine (CCLM)* has taken many steps to develop advanced research and technology in the diagnosis and monitoring of tumor cancer and to help clinicians translate it into clinical practice.

Keywords: calcitonin; procalcitonin; TgAb; thyroglobulin.

Introduction

According to SEER, thyroid cancers account for 2.3% of all new cancer cases in 2022, with a 5 year relative survival rate of 98.4% [1]. Differentiated thyroid carcinoma (DTC), a malignant proliferation of the follicular cells of the thyroid gland, accounts for 90% of all thyroid cancers and is increasing threefold each decade [2] to become the fourth most common malignancy by 2030 [3]. Despite its high prevalence among endocrine malignancies, it has an excellent prognosis (survival rate at 10 years of 80–95%) [4] and a high chance of definitive cure [5].

Differentiated thyroid carcinomas are treated with (near-) total thyroidectomy and risk-adapted radioiodine therapy. Recently, the use of high-sensitivity thyroglobulin (hsTg) assays has simplified follow-up of DTC patients and improved their quality of life [6], although Tg tests can still be affected by thyroglobulin autoantibodies (TgAb). Overall, patients with undetectable hsTg after total thyroid ablation (i.e., total thyroidectomy plus radioiodine ablation or adjuvant treatment) are optimally managed by periodic hsTg measurement, combined with selective use of imaging procedures (i.e., ultrasound, radioiodine whole body scan, positron emission tomography/computed tomography) when indicated [7]. Theoretically, serum hsTg trend remains informative in patients treated without radioiodine ablation but reliable reference values are still lacking. The evaluation of TgAb dynamic trend is recommended in patients with detectable TgAb and potentially interfering Tg results (i.e., surrogate tumor marker) [8].

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Medullary thyroid carcinoma (MTC), a neuroendocrine malignancy arising from calcitonin-producing parafollicular C cells, accounts for 1.4–5% of all thyroid malignancies [9, 10]; although it has a lower incidence, especially compared with DTC, it has increased over the past 30 years from 0.14 to 0.21 per 100,000 people [11], corresponding to a prevalence of 3.8 per 100,000 people. MTC occurs as sporadic disease in most patients while inherited forms may occur in patients with multiple endocrine neoplasia type 2 (MEN2) or familial medullary thyroid cancer (FMTC) [12]. In the context of MTC, calcitonin (CT) is the most sensitive marker for diagnosis and follow-up after surgery. Routine measurement of serum levels CT is recommended in patients with nodular thyroid disease for preoperative diagnosis of unsuspected MTC, although some concerns regarding cost-effectiveness, low specificity of the assay used to measure serum levels CT, presence of heterophile antibodies, and association of elevated serum levels CT with diseases other than MTC continue to be debated. This study, retracing the pivotal role of *CCLM* in advancing knowledge of thyroid cancer, aimed to critically review the main problems in characterizing current Tg and CT as biomarkers for diagnosis/monitoring of DTC and MTC, respectively, and to identify the advantages and challenges in using the new laboratory biomarkers in the clinical management of patients with differentiated and medullary thyroid cancer.

Methods

A comprehensive search strategy of PubMed, Web of Science, and Scopus databases was conducted from June to September 2022 with no language or time restrictions. Articles focusing on pediatric patients or considering laboratory tests other than Tg and CT for the diagnosis/monitoring of MTC and DTC were excluded *a priori*. The authors independently reviewed titles and abstracts and selected a list of potential articles to include in the study. After full-text screening, supplemental analysis of potential articles was performed using references from the included articles.

Results

Differentiated thyroid carcinoma

Thyroglobulin is a large 660 kDa glycoprotein specifically synthesized by thyroid follicular cells for thyroid hormone production. Due to its post-translational modification as well as the unregulated synthesis of mature thyroglobulin in thyroid tumor cells, it exhibits a heterogeneous structure [13]. When the thyroid gland is damaged by benign or malignant thyroid disease, Tg can enter the bloodstream. It

has a serum half-life of 65 h [14] and a serum level proportional to the amount of thyroid tissue, with 1 µg/L corresponding to 1 g [15]. Thyroglobulin is usually measured with continued T4 treatment (onT4-Tg), and an elevated level should raise suspicion of cancer recurrence. For this reason, Tg measurement is considered the gold standard for tumor detection in patients with DTC, especially after complete surgical removal of benign and malignant thyroid tissue and radioiodine (I-131) ablation/adjuvant therapy. Almost all patients with localized disease or distant metastasis have detectable or elevated Tg levels, whereas patients in stable remission have undetectable serum Tg levels even after stimulation by endogenous thyrotropin (TSH) or recombinant human TSH administration [5, 16]. Tg tests performed after surgery or immediately before radioiodine ablation have been used to identify DTC metastases and to predict the outcome of a post-treatment whole-body examination [17]. Most DTC patients become disease-free after the initial surgery, but recurrences occur in up to 5–28% [18, 19]. Since the pretest probability of disease recurrence is very low in the majority of DTC patients, follow-up protocols must have a high negative predictive value to avoid needless testing and a high positive predictive value to easily detect persistent/recurrent disease.

Over the years, we have observed a gradual increase in the sensitivity of Tg assays, starting with the original radioimmunoassays (RIAs), which indicated values of 2–5 µg/L, to the first immunometric assays (IMA) with values as low as 1 µg/L and, more recently, high-sensitive IMAs (hsTg) with Functional Sensitivity values of 0.1–0.2 µg/L [13]. Although the role of serum Tg level as a biomarker is not relevant for the diagnosis of DTC, several studies have demonstrated its utility in assessing tumor extension in confirmed DTC cases. In a Korean study conducted in a sample of 57 DTC patients, preoperative serum Tg levels reported a sensitivity of 84.2% and a specificity of 90.6% in predicting the presence of initial distant metastases (cut-off 63.4 ng/mL) [20]. Accordingly, in a large retrospective study conducted on a sample of 4,029 DTC patients, significantly higher serum Tg concentrations were found in patients with higher tumor burden and extent, such as the presence of lymph node involvement and distant metastases [21]. In contrast to these results, an American study of 598 DTC patients found that serum Tg concentrations before surgery were associated with thyroid size and tumor stage but had low sensitivity (10.3%) for detecting distant metastases [22].

After surgery, the predictive role of serum Tg level is limited in patients treated with lobectomy [23], whereas overall it is recognized as an important marker for predicting the presence of metastases and for evaluating the 12 month outcome of patients treated with total or near-thyroidectomy.

As reported in a 2005-study by Giovannella et al., serum levels Tg after total or near-total thyroidectomy are undetectable in more than 90% of patients during T4 treatment and in 80% of patients after discontinuation of T4. In a sample of 156 patients with low-risk pT1-DTC, Tg measurement only 4 weeks after surgery and before radioiodine ablation helped identify 95% of patients with PTC metastases and correctly identified 90% of disease-free patients at 12 months with a near-absolute negative predictive value of 99% (Tg less than 3.20 mg/L) [24]. In another study performed on a sample of 117 patients with histologically proven DTC treated by total thyroidectomy and radioiodine therapy, the same authors evaluated the role of onT4-Tg measurement with a high-sensitivity method in patients with low-risk DTC and compared the onT4-Tg assay with the results of the rhTSH-stimulated Tg test and with the results of neck ultrasound (US). Recurrences were detected in 14 patients, while 103 patients had no evidence of disease. onT4-Tg in serum was detectable in 10 patients with recurrences, and two patients had US-negative/onT4-Tg-positive recurrences, as well as three US-positive recurrences with undetectable onT4-Tg. The combination of onT4-Tg assay and US examination correctly identified 13 of 14 recurrence patients, with nine false-positive results in patients without signs of disease. After rhTSH stimulation, conversion from undetectable to detectable Tg was observed in six patients, two with and four without recurrence, respectively. In all patients with recurrences and detectable onT4-Tg after rhTSH administration, Tg continued to increase. The negative predictive value of onT4-Tg was 96% and reached 99% when combined with US [25]. In another study, Giovannella and colleagues examined the effects of onT4-Tg measurement on early and long-term outcomes of patients with DTC in the scenario described above. In a sample of 195 patients, seven patients had detectable onT4-Tg (0.3–1.4 ng/mL) and a suppressed TSH (<0.1 mIU/L) at first follow-up. Six months after

thyroidectomy, four patients with proven DTC recurrence and three patients without proven DTC recurrence showed a rise in serum onT4-Tg (sensitivity 100%, specificity 98%, PPV 98%, NPV 100%, accuracy 98%). Three patients who were positive at first follow-up experienced spontaneous remission within 3–6 months, so supporting that a negative Tg trend has a negative predictive value of 100%, and none of them relapsed during follow-up (3.7–7.1 years). Relapse occurred in 3/188 who were negative during the early follow-up period, and in all cases, there was a progressive increase in onT4-Tg levels. Indeed, Tg levels increased in five patients: recurrence was observed in four patients, whereas the fifth remained healthy despite increasing serum Tg concentrations, possibly due to TSH-stimulation. In 12 disease-free patients a temporary increase in onT4-Tg occurred during long-term follow-up, which however regressed spontaneously within 3–6 months [26]. A recent systematic review and meta-analysis examining the diagnostic performance of Tg in the presence of concurrent undetectable TgAb and evaluating the prognostic performance of undetectable Tg in predicting absence of disease signs during subsequent follow-up, found a significant NPV of onT4-Tg for both diagnostic and prognostic performance (Table 1).

The risk of structural disease in a patient with DTC and undetectable onT4-Tg is 0.6%. The use of an hs-Tg assay should increase the risk of false-positive results because normal thyroid remnants are more easily detected due to the higher sensitivity of the same hs-Tg assay. Approximately 1% of false-negative results, of which only one was TgAb-positive, were due to undetectable onT4-Tg in patients with structural signs of disease. TgAb positivity represents the major limitation to the reliability of stimulated and onT4-Tg. Indeed, TgAb is not detected in approximately 75% of DTC patients, but when it occurs, it can interfere with Tg determination. For this reason, it is

Table 1: Summary estimates of diagnosis and prognostic performance of ON-T4. (Table source: with permission from [27]).

Analysis	No. of patients (no. of studies)	Sensitivity (95%CI)	Specificity (95%CI)	Positive predictive value (95%CI)	Negative predictive value (95%CI)	Likelihood ratio for positive results (95%CI)	Likelihood ratio for negative results (95%CI)	Diagnostic odds ratio (95%CI)
Diagnostic performance	1568(8)	83.4% (73.4–90.1)	79.9% (63.0–90.3)	21.3% (11.9–30.7)	99.4% (98.9–99.9) ^a	4.2% (2.4–7.6)	0.2% (0.1–0.3)	18.7% (6.1–57.4%)
Prognostic performance	923(6)	86.3% (29.6–98.9)	70.7% (52.6–83.9)	7.3% (2.1–12.4)	99.4% (98.8–100) ^a	2.2% (1.2–4.1)	0.3% (0.1–1.0)	5.3% (1.6–17.8) ^b

^a I^2 less than 25%, corresponding to low heterogeneity of findings; ^b I^2 from 25 to 50%, corresponding to mild heterogeneity of findings. The likelihood ratio for positive results is the likelihood that a detectable ON-T4 hs-Tg would be expected in patient with SED compared to the likelihood that that same result would be expected in a patient with NED. A likelihood ratio between 0.2 and 5 gives no more than weak evidence; evidence; between 5 and 10, and between 0.1 and 0.2 gives moderate evidence, greater than 10 or less than 0.1 gives strong evidence. The diagnostic odds ratio describes the odds of the detectable ON-T4 hs-Tg in a patient with SED compared with the odds of the same results in a patient with NED. The value ranges from 0 to infinity.

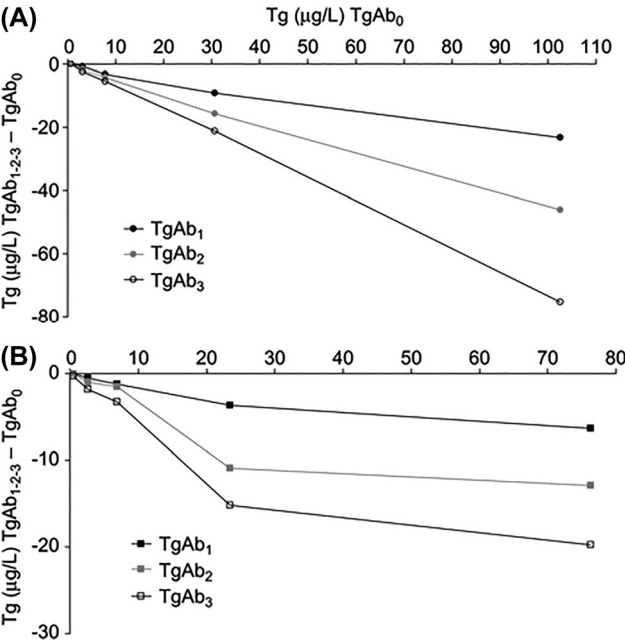


Figure 1: Effects of increasing TgAb concentrations on Tg measurements using the Immulite 2000 (A) and UniCel 80 Dxi (B) platform. (Figure source: with permission from ref. [28]).

recommended to test for TgAb positivity in conjunction with serum Tg [27].

Comparing Tg antibody interference in first- and second-generation immunoassays Tg, Giovannella et al. showed that the decrease in Tg levels in the presence of TgAb was more noticeable in the first-generation Tg assay (41.6%) than in the second-generation assay (31.4%) ($p<0.01$), respectively (Figure 1A and B) [28].

The second-generation assay showed detectable Tg concentrations in all sera and thus gave different results than the first generation assay. The discrepancies in TgAb measurement and TgAb interference in Tg measurement could be due to the different Tg/TgAb relationship (asymptotic vs. linear) and the different functional sensitivities where Tg remains fully detectable. Similar conclusions were reached by Katrangi et al. [22] who investigated the analytical and clinical comparability of different TgAb assays and found that the comparability and quantitative agreement between TgAbs was poor and that it was not possible to compare serial test results obtained with different TgAb assays. In addition, these authors demonstrated that the prediction of the presence of disease in TgAb + patients was poor with all assays (ROC AUC=0.65), suggesting that the combination of Tg and TgAb is no better than Tg alone [29]. In a recent paper examining whether different Tg and TgAb tests affect treatment recommendations for DTC patients, van Kinschot et al. [22] showed that clinical management

may vary depending on the test and that fixed cut-off values, as proposed in international guidelines, are not appropriate. Using the most informative cut-off value of 1.0 ng/mL, discrepancy was found in 6.0 and 4.3% of patients, respectively, with disagreement about response to therapy in 16.2% of patients [30]. The reasons for this discrepancy were considered to be the propensity of the different methods and differences in functional sensitivity and TgAb positivity. Lower functional sensitivity means more patients with indeterminate response at the expense of the number of patients with excellent response. This means more intensive follow-up, longer TSH suppressive therapy, and additional therapies. Therefore, test-specific cut-off values for better comparability of clinical performance [31] and tailored cut-off values for TgAb status [27] are recommended. In patients with evidence of structural disease, TgAb interference rarely resulted in undetectable Tg levels, but a small proportion of recurrent disease could be missed [30]. Artificially increasing or decreasing serum Tg levels can have significant clinical consequences, such as radiation exposure or even invasive procedures or false reassurances leading to omission of effective treatments [32]. Recovery tests have been proposed as a potential solution to reduce TgAb interference. Mean recoveries have been shown to be significantly lower in TgAb + specimens, but normal recoveries do not rule out TgAb interference [33]. In a study testing a new sensitive recovery method (Kryptor® Tg-minirecovery test) in a series of 167 DTC patients, all of whom had low or undetectable Tg levels, Giovannella et al. found high agreement between the new minirecovery (mRec) test and sensitive TgAb immunoassay results, likely due to the high sensitivity of the Tg-mRec, but no additional clinical benefit in most patients (Figure 2A and B) [34].

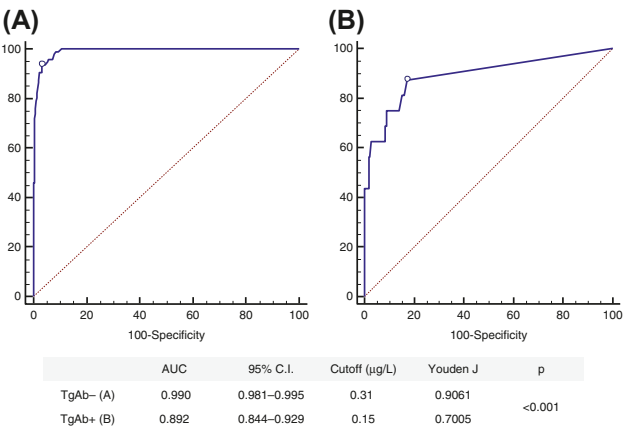


Figure 2: Serum Tg performed better in patients with negative compared to those with positive TgAb, respectively. Thyroglobulin ROC curves in DTC patients with negative (A) and positive (B) TgAb. (Figure source: with permission from ref. [34]).

While interference of TgAb on Tg measurements is well known and remains a challenge, potential problems can arise from interference of HAb with Tg measurements. In a study performed on 406 specimens after treatment with HBT tubes, five specimens (1%) were found to have HAb interference, of whom three sera showed false positive or falsely elevated Tg levels, while two revealed false negative or falsely decreased Tg levels [32]. These results contrast with those of Preissner and colleagues, who, using an automated chemiluminometric Tg assay, detected 2% false-positive or falsely elevated Tg values but no false-negative or falsely depressed Tg values [35]. Positive HAb interference should be suspected if the Tg elevation is not consistent with the clinical context, in which case the simplest approach is to repeat the test with a different assay. In contrast, negative HAb interference because false-negative results are generally difficult to detect on the basis of clinical evidence [32].

In the last decade, monitoring of dynamic changes in Tg concentration, Tg doubling time (Tg-DT), has been proposed as a valuable biomarker for predicting follow-up outcomes, independent of classical prognostic factors such as TNM or age [36]. Patients with Tg-DT <1 year (43.8% at 5 years and 78.6% at 10 years) were found to have a higher rate of locoregional recurrence than patients with Tg-DT between 1 and 3 years (23.5% at 5 years, 72.6% at 10 years) and in patients with Tg-DT ≥ 3 years (23.6% at 5 years and 42.5% at 10 years), while the risk ratio for overall survival in patients with Tg-DT <1 year ($n=247$) was 2.09 compared with those with Tg-DT ≥ 1 [37].

Medullary thyroid carcinoma

Calcitonin, a 32 amino calcium-lowering peptide secreted by the thyroid C-cell, is a sensitive tumor marker for primary diagnosis as well as for follow-up of MTC and C-cell hyperplasia [38]. CT contains an intrachain disulfide bridge between sequence positions 1 and 7 at the NH₂-terminal end of the molecule and an amide group at the COOH-terminal proline. CT (1–32) is biosynthesized from the polypeptide precursor procalcitonin (PCT), a 116 amino acid long pro-hormone with three peptide components: a 57 amino acid long sequence at the amino terminus (NPCT), a centrally located immature CT with a terminal glycine, and a 21 amino acid long CT carboxy terminus peptide 1 (CCP-1) [38, 39]. In normal clinical practice, MTC is strongly suspected in patients with a CT value > of 100 pg/mL. However, establishing normal thresholds for CT in healthy individuals and upper reference limits for men and women is challenging because commercially available tests are not comparable,

and the number of C cells and thyroid volume are different in men and women. As Cavalier and colleagues have shown, the 95th percentile value for the female population was very low, 4.0 ng/L, although 95% of “normal” patients CT had concentrations below 10 ng/L [40]. A prospective cross-sectional study conducted in 2012 by Giovanella et al. in a sample of 519 thyroid-healthy subjects to evaluate the effects of thyroid volume measured by TUS on serum concentrations of CT (measured by three different immunoassays) showed that the levels of CT were higher in men than in women for all tested immunoassays ($p<0.001$), and the mean thyroid volume in the reference population was 15.8 ± 4.2 mL in men and 9.9 ± 2.7 mL in women ($p<0.001$) [41]. As reported in a recent Cochrane systematic review and meta-analysis analyzing a sample of 72,368 patients with thyroid nodules with a median prevalence of MTC of 0.32%, CT measurement had an overall sensitivity of 83–100% and a specificity of 94–100% in detecting MTC patients, and when a cut-off value of 10 pg/mL was adopted, sensitivity and specificity increased to 100 and 97.2%, respectively [42]. Although in the healthy population and in 90% of patients with thyroid diseases other than MTC, serum concentrations of CT are below 10 pg/mL, elevated, even very high, concentrations of CT are very common in the clinical setting and may increase the risk of incorrect clinical decisions. In this case, the CT pentagastrin or calcium gluconate stimulation test was traditionally required to rule out the presence of MTC: values in the stimulated CT above 100 pg/mL with pentagastrin (no longer available) were 90% specific for the diagnosis of C-cell disease but reliable thresholds are not available for calcium gluconate test [43, 44]. Although the sensitivity and specificity of the assays have reached a high standard, as just reported, the comparability between different assays is still subject to investigation. To avoid potential risk from the use of different assays, several studies recommend that if another assay must be used in an already monitored patient, measurement of CT in the patient should not simply be from one assay to the other, but that concurrent monitoring of the two assays should occur over an appropriate period of time [38]. Although the agreement and correlation between different assays is well established, as reported in the study by Cavalier et al. [22] who found good agreement between the Liaison® Calcitonin_CT II and the Cisbio® International IRMA hCT (mean difference 0.1 ng/L, standard deviation 2.0 ng/L in the basal assay CT and a mean difference of 11.1 ± 49.3 ng/L in 45 samples treated after CT pentagastrin stimulation) [40], the interchangeability of the assays remains the major obstacle. In a study conducted by Giovanella and colleagues in 2011, aimed at evaluating the determinants of serum levels CT and establishing reference ranges for three different

immunoassays (ELSA[®]-hCT CIS Bio, IMMULITE[®] 2000 XPI Siemens Diagnostic, and LIAISON[®] CT-II DiaSorin), CIS assay found higher CT concentration than the IMMULITE assay, and the latter were in turn higher than concentrations measured with the LIAISON assay, with a poor overall agreement among them [41]. The poor comparability between assays is attributed to the different CT isoforms and fragments discovered in some patients, as well as the different antibodies used in the assays which lead to a significant discrepancy between commercial CT assays, even when standardized (WHO 89/620) [12, 45–47]: the variability among different products of the CT gene causes various circulating immunoreactive isoforms and fragments, leading to different assays and CT antibody concentrations [10]. In the clinical context, serum concentrations CT as a marker for MTC can be affected by various circumstances. Elevated CT can be caused by various cancers, bad habits such as smoking or alcohol consumption, or treatments such as proton pump inhibitor therapy. In addition, non-MTC diseases such as follicular carcinoma, goiter, and autoimmune thyroid disease may also be associated with mild to moderate CT elevation [48]. In 2012, Giovanella et al. reported two cases of false-positive calcitonin results in patients with benign goiter. In the first case, a 42-year-old female patient who developed asymptomatic thyroid enlargement was found to have a slightly elevated CT concentration (10.9 pg/mL, reference value <8 pg/mL). After thorough screening with contrasting results between cytology (nondiagnostic and negative) and CT measurement (elevated), thyroidectomy was performed, which later proved unnecessary. Subsequent CT immunostaining of permanent thyroid sections revealed normal C cells and no trace of C-cell hyperplasia, micro-MTC, or MTC. In the second case report, a 54-year-old patient with asymptomatic thyroid enlargement was found to have a CT concentration of 24.9 pg/mL (reference value in men 18 pg/mL). Despite the contrasting results of CT concentration and negative cytology, complete thyroidectomy was performed. Histologic examination revealed a benign multinodular goiter without traces of C-cell hyperplasia and without micro-MCT or MCT. After one month, the concentration of CT was undetectable (<2 pg/mL) [49]. Hypercalcitoninemia can be observed in isolated C-cell hyperplasia (CCH) associated with lymphocytic thyroiditis or microPTC [50], as well as the presence of heterophilic antibodies [51]. In a clinical case reported by Censi et al. [52], a 15-year-old patient with moderately elevated CT levels (68.8 ng/mL at the first analysis, 73.9 ng/mL at the second examination) was described, presenting a contrasting clinical picture without signs of MTC. After application of the heterophilic blocking tube procedure, the concentration of CT decreased to less than 1 pg/mL [38]. In the postoperative

period, the CT level is an excellent prognostic marker: the ATA guidelines recommend strict monitoring of basal CT levels from the third month after surgery and at intervals of 6–12 months [53]. After (near-) total thyroidectomy, patients with a serum CT level of less than 10 pg/mL are considered biochemically cured and have a 10 years-survival rate of 97.7%; while persistently elevated basal levels CT after surgery are indicative of residual, unremoved MTC. In patients with undetectable levels CT after surgery, measurements should be continued twice a year for the next 2 years, knowing that biochemical recurrence can occur in 3% of patients with a normal basal serum CT level within 7.5 years [39]. Evidence suggests that in patients whose basal CT is below 150 pg/mL after thyroidectomy, persistent or recurrent disease is almost always confined to the cervical lymph nodes [53, 54]. Levels above 150 pg/mL raise suspicion of disseminated disease, and additional imaging studies should be considered.

An interesting test for postoperative management of MTC patients is the additional measurement of carcinoembryonic antigen (CEA) in serum together with CT. Usually, both markers develop in parallel, so that their simultaneously increasing values indicate either incomplete tumor removal or disease progression [53]. In the highly unusual case where a normal postoperative serum level CT is accompanied by an elevated CEA serum level, a poorly differentiated MTC should be suspected [55]. In recent years, calcitonin doubling time has been shown to be a useful prognostic tool in MTC patients. In patients with a CT doubling time of less than 6 months, the 5 year survival rate is 25% and the 10 year survival rate is 8%, whereas in patients in whom the CT doubling time is between 6 and 24 months, the 5 year survival rate is 97% and the 10 year survival rate is 37% [53]. Just as elevated CT values can lead to incorrect diagnosis or treatment in the postoperative period, low CT values should be closely monitored and confirmed. False-negative CT in MTC patients could be due to tumor cell de-differentiation, but problems related to laboratory methods should not be ruled out. Furthermore, food intake and the pulsative secretion of the hormone can significantly influence serum CT levels [10, 56]. These fluctuations are due to the same structure of CT, a monomeric peptide of 32 amino acids formed by cleavage and posttranslational processing of ProCT, which has a concentration-dependent biphasic half-life ranging from 15 to 40 min under physiological conditions and approximately 3½ h at elevated concentrations [57]. Serum CT levels are unstable, degrade very rapidly at room temperature, and are rapidly degraded when refrigerated (23% after 12 h, 35% after 24 h, and 65% after 7 days). It is recommended to collect the blood sample in the morning after a fasting night and to

process it quickly in the centrifuge after clotting, freeze it and transport it in ice cubes.

Technological developments in laboratory testing have led to significant improvements in assessment methods. CT: the initial competitive radioimmunoassays (RIAs) have been replaced by immunoradiometric “sandwich” assays (IRMA) and, more recently, by immunoassays using non-radioisotopic enzymatic (IEMA) or luminescent (ICMA) methods. Considering the poor comparability of the different methods (e.g., values from RIA are usually 10-fold higher than those obtained with the immunometric sandwich method), the type, characteristics, and reference limits of the CT assay used should always be indicated. In recent years, many authors have shown that procalcitonin (PCT) is an alternative tumor marker for MTC, thanks to its higher sensitivity and specificity, which can be used for both diagnosis and monitoring of MTC [58]. PCT is a CT precursor protein that is present at low serum concentrations in healthy individuals and increases during systemic inflammation, infection, and sepsis. It is a very stable protein with a concentration-independent *in vivo* half-life of 20–24 h that does not require refrigeration or freezing on ice and is easier to handle in the preanalytical phase [59]. Since all commercial PCT tests use the same antibodies, a normalization of biomarker for diagnosis, therapy, and monitoring of patients with MTC can be achieved. In addition, PCT has been shown to be a good marker to detect false hypercalcitoninemia caused by heterophilic antibodies [60]. Machens et al., who compared serum levels of PCT and CT in 112 patients with MTC before initial surgery, reported similar diagnostic accuracy of PCT compared with CT and determined comparable AUC for primary tumor at thresholds of 10 (PCT: 0.94 vs. CT: 0.93) and 40 (PCT: 0.92 vs. CT: 0.84) mm, extrathyroidal extension (PCT: 0.84 vs. CT: 0.83), lymph node metastasis (PCT: 0.88; vs. CT: 0.86), and distant metastasis (PCT: 0.93 vs. CT: 0.91) [61]. A study conducted by Kratzsch et al. in 2011 examining preanalytical conditions and PCT cross-reactivity in sera from 437 patients with clinical conditions associated with hypercalcitoninemia using two fully automated CT assays (IMMULITE and Liaison) and one nonautomated CT assay (IRMA, Medipan) and comparing results with PCT (Brahms Kryptor) showed that basal PCT below 0.25 ng/mL were associated with clinical hypercalcitoninemia conditions such as proton pump inhibitor therapy, chronic kidney disease, and Hashimoto’s thyroiditis, and excluded MTC cases [62]. In 2013, Giovanella et al. evaluated the role of routine measurement PCT in a large study with a sample of 1,236 patients (674 women and 542 men). Using the Immulite®

2000 XPI platform (Siemens Healthcare Diagnostics) to measure CT and the Kryptor system (BRAHMS) to evaluate PCT, the authors reported basal serum levels CT greater than 10 pg/mL in 1.1% of patients. Two patients were found to have MTC and one CCT, whereas no traces of MTC were found in the remaining 11 patients in the subsequent clinical and histological follow-ups. Basal levels CT of more than 100 pg/mL were detected in two MTC patients, while pentagastrin – stimulation CT >100 pg/mL was detected in the two MTC and in two other cases. When the same sample was analyzed to measure PCT, PCT was elevated only in the MTC patients and was undetectable in all other cases, giving an accuracy of 100%. The same accuracy was also reported for the stimulated PCT. The study showed that levels of CT >100 pg/mL and pentagastrin-PCT >500 pg/mL were associated with 100% positive predictive value in MTC patients. No MTC was diagnosed in 12 patients with thyroid nodules and moderately elevated levels in both basal and stimulated CT. Detectable levels of PCT identified exactly two MTC patients, whereas undetectable levels of PCT identified all non-MTC cases (100% sensitivity and 100% negative predictive value) [63]. In a study conducted by Lippi et al. an acceptable correlation, albeit weak at the lower end of the PCT concentration, was found in PCT measurements performed on 176 samples using 10 fully automated commercial PCT assays [64]. Similar results were also reported in a recent large retrospective study comparing PCT and CT in a sample of 169 patients with sporadic and hereditary MTC. The 210 serum samples showed good analytical and clinical correlation between CT (analyzed with ECLIA) and the three assays from PCT (analyzed with three assays from Roche, PES, and Abbott). All three PCT assays showed strong correlation with CT across the range of measurements and good agreement between the levels of PCT in the three different assays used. In patients with minimal residual disease, PCT and CT showed strong correlation, ranging from 0.759 (PES) to 0.794 (Abbott) depending on the specific PCT array. In patients with metastases, the values of CT and PCT are significantly higher than in the minimal residual risk group, and the correlation coefficient between CT and PCT is slightly higher than in the minimal residual risk group [65]. Similar results were reported by Giovanella et al., who analyzed a serum sample from 16 patients with active MTC and 23 patients with inactive MTC, 125 patients with benign thyroid disease, and 62 patients with thyroid cancer without MTC. The authors reported five false positives on CT measurements in non-MTC patients that were instead found to be undetectable using the PCT assay (Elecsys® PCT assay) (Figure 3) [66].

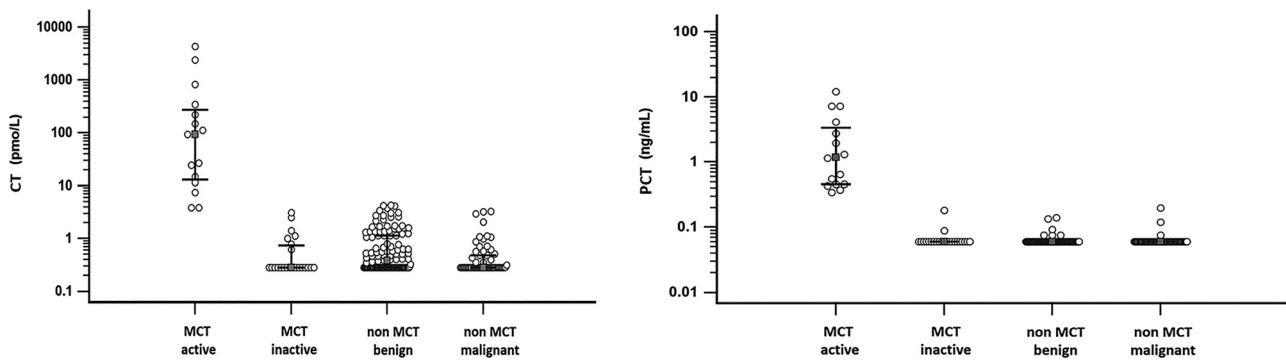


Figure 3: Distribution of serum CT and PCT in patients with active MTC, inactive MTC, non-MTC benign and non-MTC malignant thyroid disease, respectively. (Figure source: with permission from ref. [66]).

Conclusions

Timely identification of the few patients who have residual or recurrent disease is critical for the management of DTC after primary treatment. After total thyroid ablation, unstimulated hsTg measurements are adequately accurate to avoid exogenous or endogenous TSH stimulation. In addition, ultrasound monitoring should be used selectively and avoided in most patients with undetectable hsTg after therapy. As lobectomy or total thyroidectomy are increasingly performed without subsequent administration of radioiodine, robust data are urgently needed to better define the clinical interpretive criteria for Tg and TgAb in these patients, with careful consideration of serum TSH concentration and volume of remaining thyroid tissue. Normalization of Tg levels relative to TSH levels and volume of remaining thyroid tissue could establish more meaningful Tg reference ranges; however, it is extremely difficult to obtain highly accurate and reproducible Tg and TSH assays and a standardized estimate of remaining thyroid mass. Finally, in TgAb-positive patients, TgAb kinetics after therapy is a suitable “surrogate” biomarker, with decreasing TgAb levels predicting favorable outcome.

MTC is a potentially lethal tumor whose cure is highly dependent on timely diagnosis and appropriate treatment. Serum measurement of CT is the most sensitive test for early diagnosis of MTC and should be systematically performed in patients with a positive family history of MTC, CCH, or MEN2. In addition, measurement of serum CT is recommended in patients with thyroid nodules, especially in patients with features warranting FNA biopsy, with “indeterminate” cytological findings, or in patients undergoing thyroid surgery for any reason. Thanks to the availability of the new generation of CT IMAs, clinicians can avoid stimulation tests with pentagastrin or calcium gluconate, and remarkably,

recent data support the use of the PCT assay as an accurate reflex test for (mildly) elevated levels CT. In general, the development of endocrine laboratory medicine in the last decades has led to the availability of sensitive and specific biomarkers for DTC or MTC, and clinical laboratories are now in a crucial position to properly manage these patients.

Comparison of results obtained in different studies is difficult because of the different laboratory instruments and the influences of heterogeneity of Tg and CT molecules and interfering substances. As a result, Tg and CT limits are strongly influenced by the different accuracies of IMAs (i.e., differences between methods). Given the current state of the art and the insufficient harmonization of Tg and CT assays, the lack of interchangeability of laboratory results and cutoffs makes it impossible to propose and use common cutoffs for clinical practice, and the clinical laboratory unfortunately still needs to establish reliable cutoffs based on the method used. Close collaboration between clinicians and laboratory specialists is essential to translate the advantages and limitations of current assays into appropriate clinical interpretation criteria. Several *CCLM* papers over time addressed analytical and clinical issues related to biomarkers for thyroid cancer and contributed significantly to the improvement of analytical knowledge, interpretation criteria, and rational test ordering in patients with differentiated and medullary thyroid cancer. To this day, this journal remains a reference point for clinicians/scientists around the world looking for authoritative and leading opinions from the most advanced research field for daily clinical practice. For younger researchers, the topics and training methods proposed by *CCLM* represent an opportunity to develop and build new diagnostic and monitoring approaches for thyroid tumors, learn new methods, propose innovative visions, and achieve challenging goals.

Research funding: None declared.

Author contributions: All authors have accepted responsibility for the entire content of this manuscript and approved its submission.

Competing interests: Authors state no conflict of interest.

Informed consent: Not applicable.

Ethical approval: Not applicable.

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