Ther Adv Endocrinol Metab

2022, Vol. 13: 1–16

DOI: 10.1177/20420188221099344

Calcitonin: current concepts and differential diagnosis

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***Abstract*:** Calcitonin (CT) is most effectively produced by the parafollicular cells of the thyroid gland. It acts through the calcitonin receptor (CTR), a seven-transmembrane class II G-protein-coupled receptor linked to multiple signal transduction pathways with its main secretagogues being calcium and gastrin. It is clinically used mostly in the diagnosis and follow-up of medullary thyroid carcinoma (MTC). Hypercalcitoninemia can be attributed to primary (e.g. CT-secreting tumor) or secondary (e.g. due to hypercalcemia) overproduction, underexcretion (e.g. renal insufficiency), drug reaction (e.g. β-blockers), or false-positive results. In clinical practice, elevated basal calcitonin (bCT) is indicative, but not pathognomonic, of MTC. Current literature leans toward an age as well as gender-specific cutoff approach. bCT >100 pg/ml has up to 100% positive prognostic value (PPV) for MTC, whereas bCT between 8 and 100 pg/ml for adult males and 6 and 80 pg/ml for adult females should be possibly further investigated with stimulation calcitonin (sCT) tests. Calcium is showing similar efficacy with pentagastrin (Pg) sCT; however, the real value of these provocative tests has been disputed given the availability of new, highly sensitive CT immunoassays. Anyhow, evidence concludes that sCT <2 times bCT may not be suggestive of MTC, in which case, thyroid in addition to whole body workup based on clinical evaluation is further warranted. Moreover, measurement of basal and stimulated procalcitonin has been proposed as an emerging concept in this clinical scenario. Measuring bCT levels in patients with thyroid nodules as a screening tool for MTC remains another controversial topic. It has been well established, though, that bCT levels raise the sensitivity of FNAB (Fine Needle Aspiration Biopsy) and correlate with disease progression both pre- and postoperatively in this situation. There have been numerous reports about extrathyroidal neoplasms that express CT. Pancreatic, laryngeal, and lung neuroendocrine neoplasms (NENs) are most frequently associated with hypercalcitoninemia, but CT production has also been described in various other neoplasms such as duodenal, esophageal, cutaneous, and paranasal NENs as well as prostate, colon, breast, and lung non-NENs. This review outlines the current biosynthetic and physiology concepts about CT and presents up-to-date information regarding the differential diagnosis of its elevation in various clinical situations.

Keywords: algorithm, calcitonin, differential diagnosis, medullary thyroid carcinoma

Received: 12 November 2021; revised manuscript accepted: 21 April 2022.

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Introduction

Genetics and biosynthetic process

Human calcitonin (CT) is a 32-amino acid polypeptide hormone secreted mainly by the parafollicular 'C' cells of the thyroid gland, which derive from the foregut endoderm[1,2] contrary to previous reports about their neural crest origin.[3] Other tissues capable of producing CT include the lungs, small intestine, thymus, liver, parathyroid glands, as well as the liver.

CT is biosynthesized as part of a larger prohormone, called procalcitonin (ProCT). Within

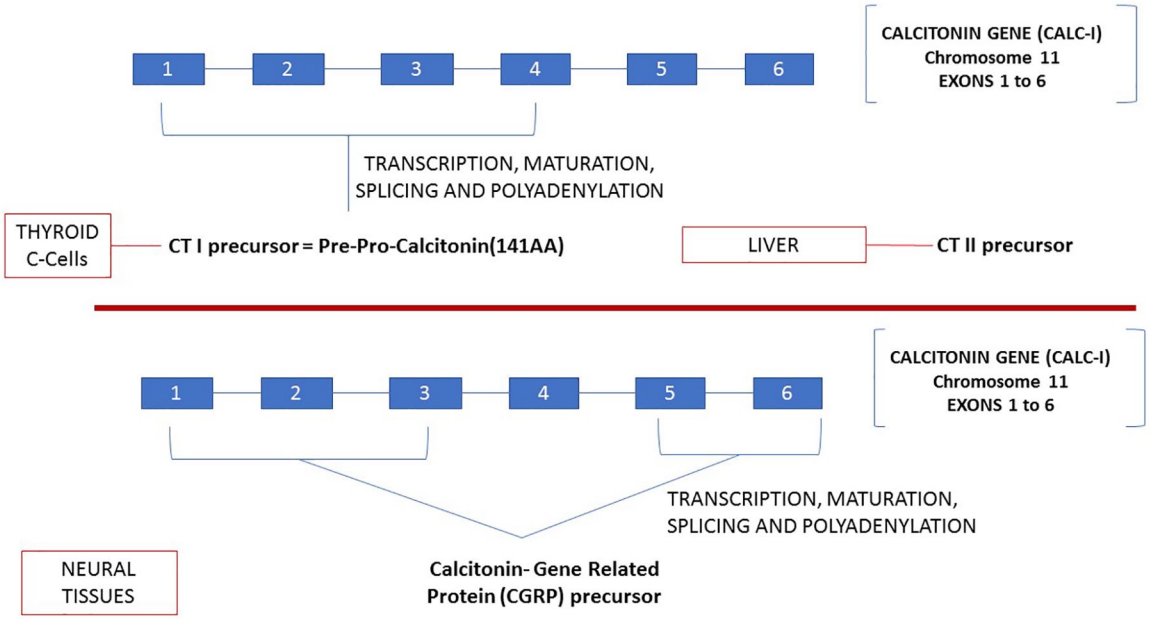

Figure 1. Tissue-specific calcitonin transcript expression: The *CALC-I* gene is processed in three mRNAs by tissue-specific alternative splicing – CT-I (exons from 1 to 4) in thyroid parafollicular cells, CT-II (exons from 1 to 3, partial 4, 5, and 6) in the liver, and CGRP-I (all exons except 4) in neural tissues.

ProCT, CT exists in a nonaminated, immature 33-amino acid form, terminated with a glycine. ProCT's posttranslational processing results in production of several additional free peptides, as well as immature CT. Further modifications of immature forms of CT end up in a protein molecule with aminated C-terminus and a disulfide bridge between its various chains.[3,4] Much of the bioactivity of the mature hormone may be linked to the amination of its carboxyl end.

Salmon-derived CT, which is used pharmacologically, differs on the amino acids 10 and 27, and thus composes a drastically different alpha helix that is more potent than its human analogue.[4]

CT: from gene to protein

Human CT is derived from *Calcitonin I* (*CALC-I*) gene located on chromosome 11p.[5] The transcript of the *CALC-I* gene subsequently undergoes tissue-specific alternative splicing: CT-I is derived mostly in the thyroid gland, whereas CT-II and CGRP-1 (Calcitonin Gene Related Protein-1), the rest two transcripts, are expressed in the liver and neural tissues, respectively (Figure 1).[6] CT-I transcription product known as pre-ProCT comprises a 141-amino acid molecule, which is then cleaved at the N-terminal to form ProCT.[7,8] ProCT contains 116 amino acids and through an amination process is finally cleaved to CT[8] (Figure 2). Mature CT contain 32 amino acids, with a disulfide bridge at the amino terminal end (between amino acid positions 1 and 7) and a proline at the carboxyterminal end. At the carboxyl terminus of the CT (1–32), the proline is aminated. Importantly, both the ring structure and this aminated proline are essential for the full expression of its known bioactions (Figure 3).

Because prohormones are not secreted in the blood stream in healthy individuals, ProCT in the serum serves as a helpful biomarker in cases of bacterial-mediated septic states. More specifically, the production of ProCT in medullary thyroid carcinoma (MTC) is mediated by the thyroid C-cells, whereas in cases of acute bacterial infection, the most probable sites are the neuroendocrine cells of lungs and intestine. Consequently, ProCT synthesis in the former case depends upon elevated calcium levels, glucocorticoids, gastrin, or β-adrenergic stimulation in contrast to the latter, in which ProCT production is linked to the presence of tumor necrosis factor (TNF) and various inflammatory cytokines such as IL (interleukin)-1, IL-2, and IL-6.[9,10]

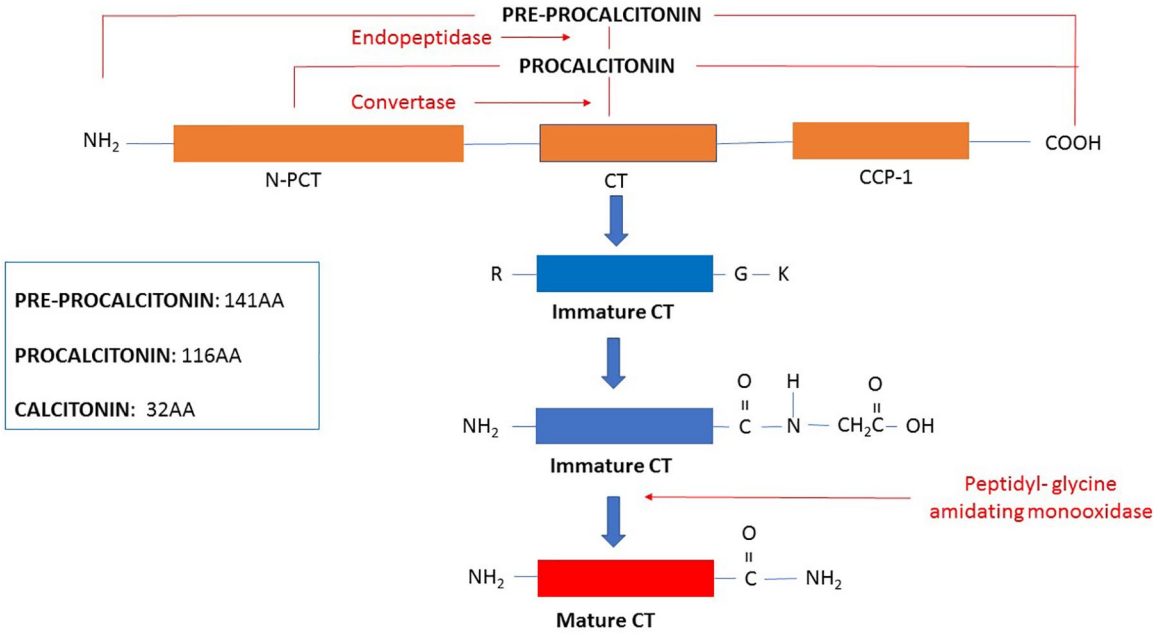

Figure 2. Biosynthetic process from preprocalcitonin to mature calcitonin. Within ProCT, CT exists in a nonaminated, immature 33-amino acid form, terminated with a glycine. ProCT's posttranslational processing results in production of several additional free peptides, as well as immature CT. Further modifications of immature forms of CT end up in a protein molecule with aminated C-terminus and a disulfide bridge between its various chains. Much of the bioactivity of the mature hormone may be linked to the amination of its carboxyl end.

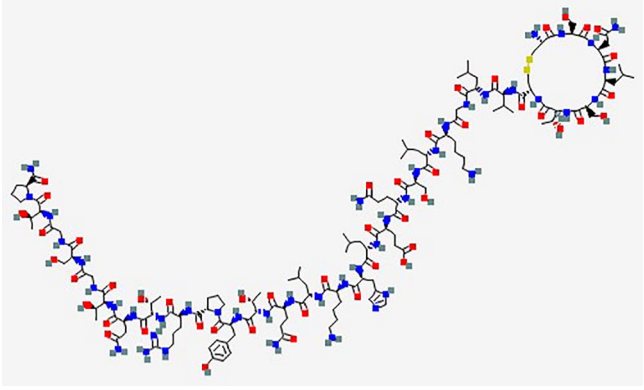

Figure 3. Mature human calcitonin is a 32-membered heterodetic cyclic peptide comprising the sequence Cys-Ser-Asn-Leu-Ser-Thr-Cys-Val-Leu-Gly-Lys-Leu-Ser-Gln-Glu-Leu-His-Lys-Leu-Gln-Thr-Tyr-Pro-Arg-Thr-Asn-Thr-Gly-Ser-Gly-Thr-Pro-NH_2, cyclized by a disulfide bridge between the two Cys residues at positions 1 and 7 and a proline at the carboxyterminal end.[85] Both the ring structure and this aminated proline are essential for the full expression of CT's known bioactions.

Healthy subjects have thus very low serum ProCT levels, which taken together with the quite long plasma half-life of 25–30 h have recently fueled a significant amount of research as to delineate the role of ProCT in MTC and sepsis.

Physiology

Even though numerous, mostly experimental, studies have linked CT with effects on blood, bone, kidneys, and respiratory, gastrointestinal, embryogenic, and central nervous system, its function in humans remains largely elusive.[3,11]

CT is implicated in calcium homeostasis; oppose the actions of parathyroid hormone (PTH) and tone down serum Ca^{2+} concentration. However, its efficacy in intervening and regulating serum calcium levels is significantly lower than other calcium-regulating hormones such as PTH and calcitriol. Moreover, in various clinical situations with high (e.g. metastatic MTC) or low (e.g. total thyroidectomy) CT serum levels, no clinical consequences have been described.

CT signaling pathways

As a peptide hormone, CT cannot cross the phospholipid membrane and thus binds to high-affinity calcitonin receptors (CTRs). The CTR is a member of a subfamily of the seven-transmembrane domain G-protein-coupled receptor superfamily that includes several peptides. Members of this family have a similar structure with other seven-membrane-spanning domain G-protein-coupled receptors.[11] Several signaling transduction pathways have been linked to CT receptor. The most important leads to upregulation of protein kinase A through the adenylyl cyclase-cAMP-PKA pathway activation (Adenosine 3,5-cyclic monoposphate Protein Kinase A).[12] Other reports suggest that phospholipase A2, C, and D can also participate in CT's second messenger cascade. Finally, another study implicated the MAPK ERK1/2 (Mitogen-Activated-Protein-Kinase Extracellular-Signal-Regulated 1/2) pathway through the Shc tyrosine phosphorylation.[13]

CT actions

Bone. Osteoclasts are the major target for the action of CT and one of the best studied. CT plays an important role in skeletal homeostasis, being a key modulator on bone resorption.[14] Acutely, the hormone acts directly to the CTR altering the osteoclast sensitivity to serum calcium. It induces quiescence of the osteoclast motility with retraction of the pseudopods along with a cessation of membrane ruffling.[15] However, CT acts for a short time frame, as osteoclasts 'escape' its function in 24–48 h.[4] CT inhibits also other components of the osteoclast such as, the release of acid phosphatase and the expression of carbonic anhydrase II, focal adhesion kinase, and osteopontin.[16] Furthermore, it interferes with osteoclast differentiation preventing osteoclast maturation. The overall impact of the osteoclastic inhibition is to decrease bone resorption.

Kidney. Another site of CT action is renal tubules. More specifically, CT promotes urinary excretion of phosphate and calcium by inhibiting reabsorption in the proximal and distal convoluted tubules.[4,11]

Other sites. In central nervous system, large doses of CT reduce serum testosterone, LH (Luteinizing Hormone), and FSH (Follicle Stimulating Hormone) levels. Chronic administration in migraine patients is associated with increased β-endorphin, ACTH (Adrenocorticotropic Hormone), and cortisol. In gastrointestinal system, high CT levels invoke water and electrolyte secretion at the jejunum and ileum, a mechanism possibly implicated in the diarrhea seen in some patients with MTC.[11]

CT secretion is mainly regulated by serum calcium and gastrin concentration, a principle that is clinically used in the calcium or pentagastrin (Pg) CT stimulation tests, respectively.

Clinical use of CT

CT can be used both in diagnostic and in therapeutic clinical situations; diagnostically, it can either be measured in the serum or stained in immunohistochemistry specimens. Elevated serum CT is not specific for any pathology, but depending on the underlying clinical circumstances, it can be relevant in inclusion and exclusion of various diagnostic possibilities. On the contrary, immunohistochemical expression of CT is a major diagnostic tool in MTC and various neuroendocrine neoplasms (NENs).

CT and MTC (preoperative setting)

Diagnostically, serum CT basal concentration is helpful in the detection of MTC, while it is debated whether it can be used also, for the differential diagnosis between MTC and C-cell hyperplasia (CCH). The latter, described as the presence within both thyroid lobes of at least one area with more than 50 C-cells in a single low-power field (magnification $\times 100$), is generally considered a precancerous condition in the familial MTC.[17,18] The CT cutoff value of 100 pg/ml has been shown to be highly predictive of MTC, whereas surgery is usually recommended above this threshold.

Furthermore, basal calcitonin (bCT) levels are indicative of metastatic potential. Patients with bCT >500 pg/ml must be initially evaluated for

distant metastases, whereas those with bCT <500 pg/ml have lower risk and may proceed to thyroidectomy, as per ATA (American Thyroid Association) guidelines.[18] Finally, bCT levels may indicate the presence and define the extent of lymph node metastasis (LNM). In a study of 300 patients with MTC treated by total thyroidectomy and compartment-oriented lymph node dissections, there was virtually no risk of LNMs when the preoperative serum CT level was <20 pg/ml.[19] Basal serum CT levels exceeding 20, 50, 200, and 500 pg/ml were associated with metastases to lymph nodes in the ipsilateral central and ipsilateral lateral neck, the contralateral central neck, the contralateral lateral neck, and the upper mediastinum, respectively.[19] In another study with 170 patients, the preoperative CT level was positively correlated with primary tumor size (rho = 0.744, $p < 0.001$) and LNM number (rho = 0.537, $p < 0.001$).[20] Preoperative CT thresholds of 20, 200, and 500 pg/ml were associated with the presence of ipsilateral lateral LNM, contralateral lateral LNM, and distant metastasis, respectively. Given these results, preoperative CT level has diagnostic value for predicting LNM, correlates with disease extent, and can be used to determine the optimal initial surgical extent.[20–22] As far as LNM is concerned, an interesting application of CT measurement includes CT detection in the washout fluid of FNA (Fine needle Aspiration) biopsy (FNA-CT) in cases of MTC LNMs.[23] The rationale behind this specific use of CT has been extrapolated from evidence showing that the diagnostic accuracy of FNA in MTC was markedly increased by IHC (ImmunoHistoChemistry) analysis of the FNA specimen and additionally by measuring CT levels in the FNA washout fluid.[24] Few recent studies have brought new data on the subject, supporting the value of FNA-CT as a reliable and inexpensive diagnostic tool, along with FNA cytology, that should be included in the clinical workup of cervical lymph nodal involvement in patients with thyroid nodules or history of MTC.[23,25] However, more studies are needed to better delineate the cutoff values and the relevance of this technique in the management of MTC patients.

On the contrary, only few 'calcitonin-negative' MTCs, in which CT was not elevated in the serum, have been described in the medical literature.[26–28] Of the CT-negative MTCs, fewer still had negative serum CT in the presence of strong, diffuse immunohistochemical staining for CT in the primary tumor,[28] and only three patients had completely undetectable levels of preoperative CT.[26] Proposed pathophysiologic mechanisms for the loss of CT production include dedifferentiation of the tumor which may imply a poor prognosis or impaired cellular secretory mechanism. In any case, CT-negative MTC remains very rare, with an estimated prevalence of 0.83%.[28]

CT and MTC (postoperative setting)

CT levels serve as an excellent prognostic marker postoperatively. bCT should be obtained 3 months after surgery and monitored every 6–12 months as per latest ATA guidelines.[18] Patients whose basal serum CT level is normal (<10 pg/ml) following attempted complete lymph node dissection are considered 'biochemically cured' and demonstrate a 97.7% survival at 10 years.[29] However, 3% of patients with a normal baseline serum CT level following thyroidectomy will ultimately exhibit biochemical recurrence within 7.5 years.[30]

Patients with undetectable serum CT postsurgery should continue measurements twice a year for the next 2 years. Persistent postoperative elevated bCT is indicative of residual, unresected MTC. The magnitude of bCT elevation in these cases is suggestive of the subsequent management and prognosis. Evidence suggests that in patients whose basal serum CT levels is less than 150 pg/ml post-thyroidectomy, persistent or recurrent disease is almost always confined to lymph nodes in the neck.[18,31] An interesting remark in the postoperative setting of MTC patients refers to the addition of serum carcinoembryonic antigen (CEA) measurements along with CT. Usually both markers evolve in parallel, so their increasing values indicate either incomplete tumor removal or disease progression.[18] In the highly unusual discordant case of normal postoperative serum CT combined with elevated serum CEA level, a poorly differentiated MTC should be suspected.[32]

CT – pharmacologic use

As a drug, CT usage has been greatly diminished in recent years. The commercially available drug is the salmon CT as a nasal or subcutaneous/intramuscular preparation. Currently, CT is FDA (Food and Drug Administration)-approved for use as a second-line treatment in patients

with postmenopausal osteoporosis and Paget disease of the bone in cases of bisphosphonate intolerance as well as in hypercalcemia.[4] Its short-term action can produce symptomatic relief both in osteoporosis and in Paget disease of the bone, while co-administration with bisphosphonates can elude the escaping mechanism of osteoclasts. CT therapy has a peak effect on osteoclasts at 24–48 h, while bisphosphonate therapy requires 3 months to maximally suppress bone resorption.[33] Short-term use of CT significantly reduces osteoporotic bone pain compared with placebo, especially in the acute setting. Therefore, CT may be a preferred treatment in cases of acute osteoporotic fracture.[34] CT is FDA-approved for the treatment of hypercalcemic emergencies.[4] Following rehydration with a saline solution, co-administration of a bisphosphonate and CT at a dose of 4 IU/kg every 12 h lowers calcium effectively. After 24–48 h, the osteoclasts partially escape the action of CT. As bisphosphonates reach effective dosages after 48 h, their activity ramps up as CT's activity declines. CT can be also given with other calcium-lowering drugs in this setting, including loop diuretics, oral phosphate, and corticosteroids.[35,36]

CT measurement assays

Several different assays have been used to measure serum CT levels. First-generation radio immunoassay (RIA) was inaccurate, because it made use of polyclonal antibodies that detected various CT isoforms or CT-like proteins.[37] RIA, now of historical interest, has been replaced by two-sided immune radiometric assays (IRMAs), which use double antibodies, able to bind to two different epitopes within the CT molecule. Further progress resulted in the use of fluorescent IFMA (ImmunoFluorometric assay)[38] and chemiluminescenttests(ICMA–ImmunoChemiluminometric Assay), which are even more sensitive and specific for CT, lowering the limit of detection in 1 pg/ml.[39] The electrochemiluminescence immunoassay (ECLIA) is the most promising recent method, which using streptavidin–biotin technology exhibits a shorter test time and a low detection limit $<$1 pg/ml.[39] Nevertheless, these modern immunochemiluminometric assays, being more sensitive and specific in terms of monomeric CT detection, must prove their role regarding two major diagnostic dilemmas on the subject; the differential diagnosis between CCH and micro-MTC and the exclusion of ectopic CT production of NENs.

CT stimulating tests

CT stimulating tests have been described either using calcium or Pg. Pg test, once considered as the best, most rapid, easiest applicable test for the diagnosis of early MTC, is currently abandoned due to unavailability of Pg. Consequently, calcium stimulation test, a long forgotten provocative test for nearly 30 years, has been reinstituted and is currently the 'new standard'.[40–42] Clinically, this test has low cost and exhibits a safe profile with few adverse reactions mainly as a feeling of warmth, nausea, flushing, headache, paresthesia in the extremities or lips, abdominal cramping, and urinary urgency.[40]

The rationale behind its use, and of the stimulating tests in general, is the theoretical improvement in the diagnostic accuracy between MTC *versus* other C- or non-C-cell diseases in cases with low or moderate CT elevation. However, no widely accepted cutoff values do exist, so in clinical practice, an overlap of CCH and MTC still take place. Current ATA guidelines recommend use of individual center cutoff values for the interpretation of basal and stimulated CT values. Due to this fact, instead of assessing absolute poststimulating CT values, others have proposed the use of times increase above the basal level. It was thus proposed that the level of increasing of stimulated CT with respect to the basal value is of greater importance, because in MTC it is usually greater than 3–4 times the basal value.[43]

Elevated serum CT levels: causes and controversies

Overproduction

Endogenous CT secretion can be increased as a physiologic consequence of excessive stimuli, such as hypercalcemia and hypergastrinemia. Primary hyperparathyroidism and malignancy are the most common causes of hypercalcemia, accounting for ~90% of cases.[44] However, chronic hypercalcemia is a rare cause of CT elevation. Oral calcium administration elicits diverse hormonal response in these cases that helps in the differential diagnosis of these conditions.[45,46] Thyroid parafollicular cells can express calcium-sensing receptor like the one expressed on the parathyroid gland. In contrast to PTH, though, binding of the receptor stimulates CT release. Hypergastrinemia can be caused by achlorhydria

(e.g. pernicious anemia, atrophic gastritis, antacids) or Zollinger–Ellison syndrome.[47,48] Finally, CT can be elevated in various situations in which its precursors are elevated, such as pancreatitis, bacterial inflammation, sepsis, or CGRP inhibitors (migraine treatment).[49,50]

Overproduction of CT can also be caused primarily and not as a response to CT secretagogues. While not pathognomonic, elevated CT is mostly associated with MTC and serves as a diagnostic and prognostic tool as described in the previous chapters. However, MTC is not the only thyroid pathology that can present with hypercalcitoninemia (Table 1).

CCH[17] has been associated with slight increases of both basal (10–30 mg/dl) and stimulated serum CT levels (<560 mg/dl). CCH derives its clinical significance upon whether it constitutes a premalignant condition or not. CCH has been found in up to 33% in autopsy studies of subjects without known thyroid disorders and is twice more common in men than women. More frequently, it may be found in older patients and in those with coexisting hyperparathyroidism, hypergastrinemia, and autoimmune thyroiditis. Interestingly enough, CCH has been described in proximity to follicular-derived thyroid tumors, especially malignant ones.[51] Significantly, CCH that occurs secondarily after hyperparathyroidism, chronic autoimmune thyroiditis, renal insufficiency, and aging is not considered premalignant.[18] Conversely, CCH that occurs in hereditary MTC precedes the development of MTC and carries a definite neoplastic potential.[18]

Chronic autoimmune thyroiditis has been linked to elevated CT levels with conflicting results. C-cell damage or adjacent CCH may constitute possible mechanisms of elevated serum CT levels in some cases;[52] however, most recent studies failed to identify any such association.[53–55]

Sporadic cases of multinodular goiters have been also associated with increased CT levels. The question of whether to perform a routine measurement of serum CT in all cases of thyroid nodules has been a matter of debate and controversial guidelines between American[18] and European societies.[43,56–59] Numerous studies have tried to determine the exact role of routine CT measurement in cases of thyroid nodular disease.[56–61] All studies suggest that screening of thyroid nodules with serum CT measurement allows the diagnosis and treatment of MTC at an earlier stage, resulting in a better outcome compared with MTC not detected by serum CT measurement. One of the reasons for this finding is that increasing the preoperative diagnostic accuracy of MTC prompts the surgeon to perform a more radical and possibly curative treatment. On this basis, routine measurement of basal serum CT levels should be considered an integral part of the diagnostic evaluation of thyroid nodules. In the most recent meta-analysis on the subject, routine serum CT measurement in the management of patients with thyroid nodules has been proven valuable for the detection of medullary thyroid cancer. However, the study insists on interpreting the published cutoff values under the context of individual's Center experience.[62] Moreover, given that the prevalence of MTC in patients with thyroid nodules is 0.30–1.4%, few studies dealt with the cost-effectiveness issue of routine CT measurement. Should we measure CT in all thyroid nodules or preferentially in certain patient subgroups with a higher probability of MTC? Relevant patient subgroups may comprise patients with nodules harboring suspicious U/S features and/or indeterminate cytologic findings, patients with nodules located in the upper/middle thyroid regions, or even perhaps patients with tender nodules on palpation.

A 2008 USA study demonstrated that routine CT screening in the evaluation of patients with thyroid nodules could be performed with cost-effectiveness comparable with other widely accepted screening programs.[63] In addition, CT screening appeared to be more cost-effective in young men with larger thyroid nodules. A most recent study from USA showed that routine CT screening is a cost-effective strategy if the cost is less than $236.03. However, even though the study raised some doubts about the ability of routine CT screening to detect MTC at early stages, it suggested that it is a cost-effective alternative of discovering MTC after thyroid lobectomy or on follow-up of a thyroid nodule.[64]

So far, current ATA guidelines cannot recommend either for or against routine measurement of serum CT (recommendation rating: I). This level of rating indicates that the evidence is insufficient to recommend for or against, because either evidence is lacking that the serum CT measurement in all thyroid nodules improves

Table 1. Causes and clinical relevance of hypercalcitoninemia relative to bCT and stimulated serum CT values.

Clinical causes of ↑bCT	Relative serum bCT levels	Ca++ stimulating test
Medullary thyroid carcinoma	Low to high	Variable: + to ++
C-cell hyperplasia	Low to moderate	+
Nonthyroid nonneoplastic diseases		
Hypercalcemia (acute)	Low	–
Increased serum gastrin		
Pernicious anemia		
Chronic atrophic gastritis		
Zollinger–Ellison syndrome		
Chronic renal failure		
Cirrhosis		
Pancreatitis		
Inflammatory conditions		
Sepsis		
Nonthyroid neoplasms	Low	–
NENs (PHEO/PPGL, GEP-NENs, laryngeal NENs, SCLC)		
Prostate cancer		
Breast cancer		
Drugs		–
PPIs, β-blockers, corticosteroids, glucagon, enteroglucagon, CGRP inhibitors		
Heterophilic antibodies	–	–
Macrocalcitonin	–	–
Other		
Male gender		
Smoking		
Acute alcohol consumption		
Children <3 years of age, especially <6 months		

bCT, basal calcitonin; CT, calcitonin; GEP-NEN, gastroenteropancreatic neuroendocrine neoplasm; PHEO/PPGL, pheochromocytoma/paraganglioma; PPIs, Proton Pump Inhibitors; SCLC, small cell lung cancer.

important health outcomes, or the evidence is of poor quality or even conflicting. Updated guidelines issued by the American Association of Clinical Endocrinologists, the American College of Endocrinology, and the Associazione Medici Endocrinologi from 2016 do not recommend

either in favor of or against the routine determination of serum CT level in the evaluation of thyroid nodules except in cases of nodules with suspicious U/S findings or indeterminate cytologic findings.[65]

While secretion of CT from non-MTC neoplasms is quite rare, especially in the setting of thyroid nodular disease, both NENs and non-NEN tumors have been associated with CT production. Pancreatic, laryngeal, and lung NENs are most frequently associated with hypercalcitoninemia, but CT secretion has also been described in duodenal, esophageal, cutaneous, and paranasal NENs.[3] Furthermore, prostate, colon, breast, as well as lung non-NEN have been associated with increasing serum CT levels.[3] CT-secreting extrathyroid NENs characteristically do not respond to the stimulating tests, a feature that is used as a rationale in the differential diagnosis of hypercalcitoninemia. Moreover, non-MTC diseases and conditions usually cause relatively mild bCT and stimulated calcium CT elevations compared with MTC.

Underexcretion, drugs, and measurement methodology

Serum CT can also be elevated for reasons other than overproduction. Renal insufficiency can lead to decreased clearance of CT and other hormones such as prolactin – the main difference being that, elevated CT levels do not present with clinical manifestations.[66,67] Drugs can also elevate CT levels, either by acting as direct or indirect secretagogues. These drugs include the PPIs, antacids, and CGRP inhibitors as well as β-blockers, glucocorticoids, glucagon, and CCK (Cholecystokinin).[68,69] Finally, false-positive hypercalcitoninemia can be a result of altered results. Heterophile antibodies can tamper with CT levels when measured with older ICMAs. This issue has been addressed by using modern two-site assays such as ICMA or IFMA, which maximize the affinity for monomeric CT and minimize cross-reactivity with heterophile antibodies.

Approaching elevated serum CT

In clinical practice, elevated serum CT should always raise suspicions for MTC. This also means that patients with hypercalcitoninemia should be thoroughly investigated for other possible diagnoses whenever the clinical context appears equivocal. While approximately 5% of thyroid nodules are associated with elevated CT, only 10–40% are eventually diagnosed with MTC.[70] Moreover, in the clinical situation of workup of thyroid nodules, elevated CT caused by extrathyroidal neoplasms is rarely seen. If hypercalcitoninemia is discovered incidentally, and history or physical examination does not suggest an underlying cause, a complete thyroid nodule workup should be performed.

CT cutoff levels

One important factor when dealing with hypercalcitoninemia could be its magnitude and its association with different diagnoses. A 5817-patient cohort study suggested that bCT levels may be indicative of MTC likelihood in patients with a thyroid nodule. Patients with bCT >100 pg/ml were 100% diagnosed with MTC, while respective percentages in patients with bCT between 50 and 100 pg/ml were 25% and in patients with bCT <25 pg/ml, 8.3%. This study has limitations, as only 22 patients were diagnosed with either MTC or CCH, making it hard to set specific workup algorithms based on bCT.[71] However, one could make a case that patients with bCT >100 and >80 pg/ml for males and females, respectively, could omit confirmational stimulating testing, as, per ATA guidelines, these levels are considered highly suspicious of MTC.[18] This is also supported by a 149-patient study which suggests that with modern ICMAs, gender-specific cutoffs of >100 pg/ml for males and >85 pg/ml for females do not require stimulation calcitonin (sCT) to establish MTC (Figure 4).[72] In addition, positive prognostic value (PPV) of bCT for MTC cases with bCT >100 pg/ml in the Costante *et al.*[71] study was 100%.

CT stimulating tests

When assessing stimulated CT tests in patients, consideration should be given to the efficacy of Pg and Calcium sCT, as well as to the specific gender cutoffs. As Pg is now unavailable, interest has shifted in the evaluation of calcium stimulation test. In an older study, Niederle *et al.* proved that with specific gender cutoffs, calcium could replace Pg with nonstatistically significant

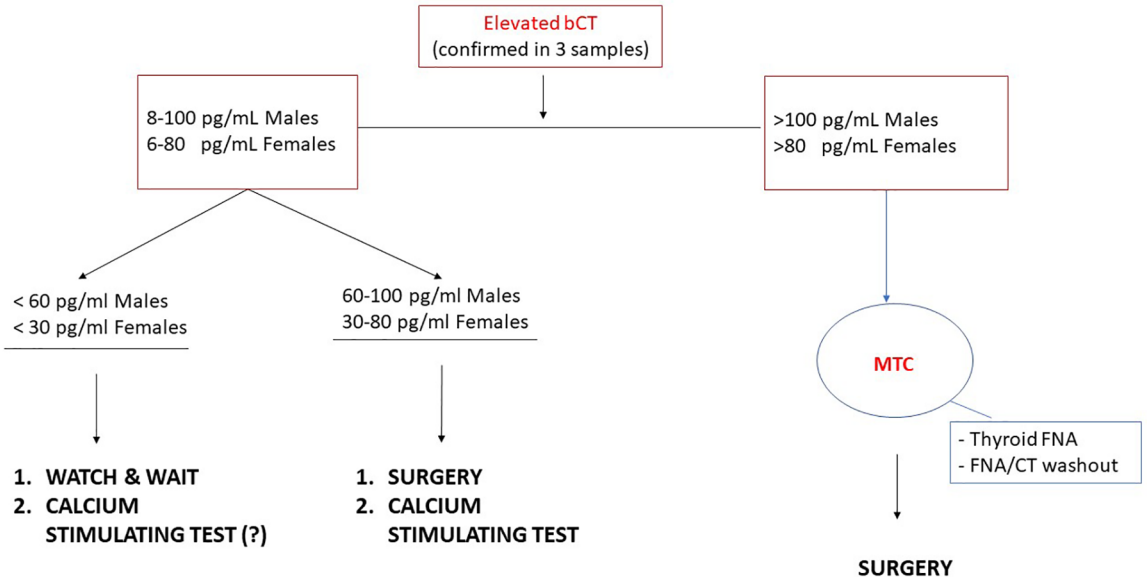

Figure 4. Proposed algorithm for the evaluation of increased calcitonin levels. The development of new assays with higher sensitivity and specificity and the definition of age- and gender-specific cutoffs have improved the diagnostic value of basal CT in predicting MTC, especially in the slightly elevated range. Non-MTC diseases and conditions usually cause relatively slight CT elevations compared with MTC. The use of CT stimulation tests decreases because of the lack of valid stimulated calcitonin values, the missing availability of pentagastrin, and the side effects of calcium infusion.

difference in diagnostic power. The study included 62 patients, of which 63% (62% males, 65% females) were diagnosed with MTC, while 37% (38% males, 35% females) with CCH. Regardless of gender, the median of maximum sCT release was higher with Ca^2+ than with Pg stimulation ($p < 0.001$), while there was strong correlation between maximum of Ca- and Pg-sCT ($r = 0.90$, $p < 0.001$).[38] Similarly, Costante *et al.*[71] proved that there is strong correlation of levels with their PPV; MTC was 100% predicted in males with bCT values >43 pg/ml or sCT concentrations >470 pg/ml (Pg-sCT) or >1500 pg/ml (Ca-sCT), and in females with bCT concentrations >23 pg/ml or sCT concentrations >200 pg/ml (Pg-sCT) or >780 pg/ml (Ca-sCT), respectively. Pg-sCT correctly predicted MTC in 16 (0.66) compared with 13 (0.54) by Ca-sCT in males (Fisher's exact test; $p = 0.556$) and in 12 (0.80) compared with 11 (0.73) in females (Fisher's exact test; $p = 1.000$). While cutoff values for sCT vary from study to study, evidence concludes that sCT <2 times bCT is not suggestive of MTC. Current ATA guidelines suggest that every center should set their own cutoff values based on experience. In addition, sCT should be repeated as necessary depending on the clinician's judgment and interpretation of the results.

However, most recent data have disputed the clinical value of the stimulating tests. More specifically, under the light of novel immunochemiluminometric assays, which are highly sensitive and specific for monomeric CT and avoid cross-reactivity, new data have become available on the subject. In a study with 91 patients, the gender-specific bCT and sCT cutoffs for the identification of MTC were >26 and >68 for bCT and >79 and >544 pg/ml for sCT in females and males, respectively. However, the bCT and sCT were found to have a similar accuracy, indicating that serum CT assays with improved functional sensitivity may likely decrease the relevance of the stimulation test in several conditions.[72] In another surgical series with 2733 patients, MTC was always present in patients with a bCT of 60 pg/ml or greater, whereas the Pg test gave no additional diagnostic information for the management of patients with elevated preoperative basal serum CT level.[73]

In the most recent study with 149 patients, it was concluded that predefined sex-specific bCT cutoff levels were helpful for the early detection of

MTC and for predicting lateral neck LNM. Importantly, the Ca-sCT did not improve preoperative diagnostics of MTC. bCT levels >43 and >100 pg/ml for males and of >23 and >85 pg/ml for females are relevant for advising patients and planning the extent of surgery.[74]

Most interestingly, an emerging role of ProCT in the diagnostics of MTC has been recently introduced by few studies. ProCT is a well-known biomarker for severe infection and bacterial sepsis; however, superior preanalytical and analytical performance along with a longer half-life compared with CT set forth its real value in MTC. A study dating back to 2011 has found that ProCT assessment may be helpful in the diagnostic workup of increased CT concentrations in questionable clinical circumstances.[75] More recently, in 2705 patients, ProCT measurement was found to be a sensitive and accurate method for detecting MTC in patients with thyroid nodules and can thus be a reliable alternative to CT measurement. Serum ProCT levels were significantly higher in patients with MTC (median = 0.64 µg/l, range = 0.16–12.9 µg/l) than in those without (median = 0.075 µg/l, range = 0.075–0.16 µg/L) ($p < 0.0001$). Overall, in 369 patients with negative preoperative PCT who underwent surgery, none of them had histological and/or immunohistochemical evidence of MTC or CCH.[76] Another multicenter study suggested that basal ProCT can be a good adjunct to CT for MTC diagnostic purposes. Given PCT's high specificity, it can be used in combination with CT in MTC diagnostics, particularly in the case of mildly elevated bCT levels. Interestingly, this study did used ProCT stimulation test and found that an sProCT level >0.19 ng/ml was able to identify MTC (sensitivity: 90.0%, specificity: 100.0%, PPV (Positive Predictive Value): 100.0%, NPV (Negative predictive Value): 86.7%; $p < 0.01$).[77] However, these studies have been carried out on limited series of MTC or non-C cell nodular thyroid diseases, thus making difficult to define the real accuracy of ProTC in terms of sensitivity and specificity in the diagnosis of MTC.

Finally, a study of 169 consecutive MTC patients found that PCT measured with three different immunoassays is as good as the standard tumor marker (CT) in the follow-up of MTC, albeit with a superior analytical stability.[78]

CT in non-MTC neoplasms

There have been numerous reports about extrathyroidal neoplasms that express CT. However, in the clinical situation of workup of thyroid nodules, elevated CT caused by extrathyroidal neoplasms is rarely seen.[61] Prostate cancer as well as benign prostate hyperplasia have been associated with elevated CT levels. A 42-radical prostatectomy specimen study showed that CT can be expressed both in malignant and in normal prostatic tissue. Prostate cancer CT levels were markedly higher in comparison with Benign Prostate Hyperplasia.[79,80] In addition, prostate cancers with higher CT expression exhibit a more aggressive course associated with distant metastases and worse prognosis overall. The activation of CT-CTR axis leads to a large increase in adherence to collagen and a remarkable increase of CD44 and CD133 in prostate cancer cells. Mutations in CTR reduce the metastatic potential of the cells expressing it, indicating the strong importance of the CT-CTR pathway in prostate cancer tumorigenesis and metastatic capability.[79]

Larynx is the next most relevant extrathyroidal anatomic structure capable of producing a CT-secreting tumor. Due to the anatomic proximity, one could certainly make the argument that CT-secreting laryngeal tumors could in fact be metastatic MTCs.[3] This theory is reinforced by the fact that both MTC and CT-secreting laryngeal cancer are associated with TFF1 (Trefoil Factor 1) and amyloid protein, as well as CT immunoreactivity. However, laryngeal NENs arise mainly from the aryepiglottic area in contrast to MTC that is supraglottis-sparing.[81]

Finally, other locations of NENs can produce CT-secreting tumors. Some pancreatic NENs (pNENs) have been associated with incidental discoveries of excessively high bCT levels.[82] CT is molecularly like insulin and as a result, overexpression of CT in NENs is almost always associated with hyperinsulinemia. In a 229-patient study, in which all were diagnosed with pNENs, CT-secreting tumors (25 patients) showed no statistically significant difference both clinically and pathologically when compared with other pNENs.[82] Finally, CT immunoreactivity can be observed in 25–43% of pheochromocytomas, but no significant clinical correlations have been discovered.[83]

Discussion

This review outlines the current biosynthetic and physiology concepts about CT and presents up-to-date information regarding the differential diagnosis of its elevation in various clinical situations. CT measurement as a diagnostic marker has not been without problems, mainly because of inaccuracies inserted by the detection methods, as well as because of lack of widely accepted age and gender cutoff bCT values. Only recently, advances in the various CT measurement assays allowed more precise estimation of CT cutoff points especially regarding MTC diagnosis. However, a gray zone area still exists, in which the differentiation mainly between MTC and CCH or other rare causes of increased serum CT levels set the basis for the clinical use of Pg or calcium stimulation tests. Recently, however, the stimulating tests have been disputed. Consequently, their use in clinical practice currently declines, because of lack of valid stimulated CT values, the missing availability of Pg, and the side effects of calcium infusion. An emerging role of ProCT has been well described in the recent literature that allows for better diagnostics of MTC.

If elevated CT is incidentally discovered, all efforts should focus on the exclusion of MTC. Renal insufficiency, acute hypercalcemia, hypergastrinemia, or drug adverse effect may be easily excluded by thorough clinical evaluation of the patient. The subsequent diagnostic steps depend on the bCT serum levels. bCT >100 pg/ml is indicative of MTC, whereas CCH or CT-secreting NENs are associated with mild CT elevation and blunted or no response to stimulating test. In any case, CT-secreting NENs are a rare clinical occurrence in cases of incidental CT elevation. bCT values between 60 and 100 pg/ml for males and 30 and 80 pg/ml for females using the latest ICMAs should still imply MTC, until proven otherwise. bCT values <60 pg/ml for males and <30 pg/ml for females may be followed in a watch and wait strategy or undertake calcium stimulation test. In case hypercalcitoninemia cannot attributed to an existing thyroid pathology, proof of extrathyroidal etiology must include detailed clinical re-evaluation and appropriate laboratory and imaging modalities.

In the presence of a relevant diagnosis, CT has a well-established role for the management and prognosis of MTC. The case of a bCT-based treatment strategy, as well as postoperative follow-up, has been fully established for MTC by the ATA guidelines.[18] In addition, CT has proven to be significant in altering the prognosis and therapy response of prostate cancer. In the future, CT suppressive therapy may prove to have a role in advanced prostate cancer cases. While many other neoplasms are associated with elevated bCT, there is no evidence-based proof that CT has an important role.

Conclusion

Despite its limitations regarding the detection assays, the cutoff values, and the protocols of the stimulating tests, CT comprises a very important tool in current endocrinology practice. It serves as a diagnostic, treating, and prognostic modality. The development of new assays with higher sensitivity and specificity and the definition of age- and gender-specific cutoffs have improved the diagnostic value of bCT in predicting MTC, especially in the slightly elevated range. Given these advances, there is increasing evidence to support more specific guidelines on how to assess different values of CT elevation. However, there is still a distinctive lack of high-level evidence data to offer specific guidelines in the 'gray zone area' that is of mild CT elevation. A serum CT cutoff value higher than the reference range may better help to discriminate real MTC from other conditions causing hypercalcitoninemia. Non-MTC diseases and NENs usually cause relatively slight CT elevations compared with MTC. Moreover, CT-secreting extrathyroid NENs characteristically do not respond or exhibit a blunted response to the calcium stimulation test.

Recently, the use of stimulating tests decreases because of the lack of valid stimulated CT values, the missing availability of Pg, and the side effects of calcium infusion. Nevertheless, differentiation between C and non-C-cell CT elevation causes should be always kept in the mind of the attending physician. An emerging role of PCT in the diagnostics of MTC appears to be promising.

Acknowledgements

None.

Ethics approval and consent to participate

N/A

Consent for publication
N/A

Author contribution(s)
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Funding
The authors received no financial support for the research, authorship, and/or publication of this article.

Conflict of interest statement
The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Availability of data and materials
Availability of data and materials can be provided upon reasonable inquiries

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