ENDOCRINE TUMOURS

Calcitonin in thyroid and extra-thyroid neuroendocrine neoplasms: the two-faced Janus

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†(Details of the Nike group are presented in the Acknowledgements section)

Abstract

An increased calcitonin serum level is suggestive of a medullary thyroid cancer (MTC), but is not pathognomonic. The possibility of false positives or other calcitonin-secreting neuroendocrine neoplasms (NENs) should be considered. Serum calcitonin levels are generally assessed by immunoradiometric and chemiluminescent assays with high sensitivity and specificity; however, slightly moderately elevated levels could be attributable to various confounding factors. Calcitonin values >100 pg/mL are strongly suspicious of malignancy, whereas in patients with moderately elevated values (10–100 pg/mL) a stimulation test may be applied to improve diagnostic accuracy. Although the standard protocol and the best gender-specific cut-offs for calcium-stimulated calcitonin are still controversial, the fold of the calcitonin increase after stimulation seems to be more reliable. Patients with MTC show stimulated calcitonin values at least three to four times higher than the basal values, whereas calcitonin-secreting NENs can be distinguished from a C-cell disease by the absence of or < two-fold response to stimulation. The measurement of calcitonin in fine-needle aspirate washout (FNA-CT) and calcitonin immunocytochemical staining from thyroid nodules are ancillary methods that may significantly improve MTC diagnosis. The present review examines the gray areas in the interpretation of calcitonin measurement in order to provide a tool to clarify the origin of calcitonin secretion and differentiate the behavior of the two-faced Janus of neuroendocrinology: intra-thyroid (MTC) and extra-thyroid NENs.

Introduction

Calcitonin is produced by parafollicular C cells located mainly in the thyroid, but also present in the lung, bladder, small intestine, liver, thymus and parathyroid glands (1). An elevated calcitonin serum level, while not a pathognomonic sign (2), suggests a medullary thyroid cancer (MTC). However, renal insufficiency,
hyperparathyroidism, neuroendocrine neoplasms (NENs) and non-neuroendocrine carcinomas (lung, colon, breast, and prostate carcinomas) as well as various drugs can increase serum calcitonin levels (3). NENs often express peptide(s) or amine(s), especially insulin, gastrin, and serotonin. Calcitonin-secreting NENs have been described in various organs including the lung, pancreas, larynx, bladder and ovaries (3, 4, 5, 6, 7, 8). Their frequency may be underestimated, as serum calcitonin measurement or immunohistochemical staining for calcitonin is not routinely performed on NENs of various origins, and even when calcitonin serum levels are measured, an elevated level may be misinterpreted as MTC, especially in patients with thyroid nodules, leading to unnecessary thyroidectomies.

We performed an up-to-date critical review taking into account the results of published studies on calcitonin measurement in both thyroid and extra-thyroidal NENs. The primary search was carried out via PubMed, EMBASE, and the Cochrane Library (until April 2020), while other articles and guidelines were retrieved from related papers or those referenced in these papers. The search was restricted to reports published in English.

The ontology of calcitonin

Origins

Calcitonin was named for its role in maintaining normal calcium tone, first described in 1962 by Douglas Harold Copp and B. Cheney (9). It was initially thought to originate from the parathyroid gland, but was later realized to be secreted by parafollicular cells of the thyroid gland (10). According to current understanding, parafollicular cells are part of the neuroendocrine system and originate from primordial C cells of the neural crest (11). These cells move to the lower pharyngeal arch, reaching the ultimobranchial bodies, and colonize the middle third of each lateral lobe of the thyroid gland. They are typically found scattered within thyroid follicles, lying inside the basement membrane but not reaching the follicular lumen, known as an intrafollicular position. Occasionally, they occur in clusters in the interfollicular connective tissue stroma, called a parafollicular/interfollicular position. Contrary to current understanding, new findings suggest that neuroendocrine cells of the mammalian thyroid gland are derived from foregut endoderm (12). This discovery adds weight to the argument that MTC should be reclassified to the family of NENs of endodermal origin (12).

Calcitonin secretion and main functions

Calcitonin is a 32 amino acid polypeptide hormone composed by a disulfide bridge and an amidated C-terminus. Its release is stimulated by elevated serum calcium and gastrin secretion (13). It acts through the calcitonin receptor (CTR), a seven-transmembrane class II G protein-coupled receptor linked to multiple signal transduction pathways (14). One of the most important pathways is coupled with adenylyl cyclase-cAMP-PKA signal transduction. However, CTRs are also coupled to the phospholipase A2, C, and D enzyme pathways. These can be activated by the coupling of CTRs to multiple G-proteins, generating the release of Ca$^{2+}$ from intracellular stores (15). Chen et al. demonstrated that calcitonin bound to CTRs stimulates Shc phosphorylation and Erk1/2 activation by parallel G$_r$- and protein kinase C-dependent mechanisms (16).

Although the biological function of calcitonin has been well established in animals such as fish, reptiles and birds, the same is not true in humans. Neither high (as in metastatic medullary thyroid cancer) nor low values (as after total thyroidectomy) produce any clinical effects in humans, and the biological role of human calcitonin remains elusive. In contrast, in in vitro experimental models, calcitonin exerts important physiologic effects on the tubular epithelium of the kidney and on osteoclasts. It reduces serum calcium and phosphate levels, acting in the tubules by promoting diuresis and decreasing resorption. In bone, it has been demonstrated to impair osteoclast function and consequently reduce both bone resorption and serum calcium levels through three main mechanisms: reduced osteoclast motility, inhibition of carbonic anhydrase II – a key enzyme in bone resorption – and prevention of osteoclast precursor differentiation into their mature form. The effect on bone is temporary; in fact, osteoclasts retract and ‘escape’ the effects of continuously administered calcitonin within 24–48 h (14, 17). The efficacy of calcitonin in calcium regulation is lower than that of other antagonist calcium-regulating hormones such as calcitriol and parathyroid hormone.

Types of calcitonin, pro-calcitonin and molecular aspects miRNAs

Mature human calcitonin originates from the calcitonin-I (CALC-I) gene, one of four genes (from CALC-I to CALC-IV) with nucleotide sequence homologies (18). The transcript of the CALC-I gene, located on the short arm of chromosome 11, generates mRNA that contain six exons,
calcitonin precursor and CGRP-I (17). 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 (19). The polypeptide CT-I precursor, known as pre-procalcitonin (Pre-PCT), is a peptide comprising 141 amino acids. Early in post-translational processing Pre-PCT is cleaved by an endopeptidase in the amino terminus region after the first 25 amino acids, resulting in a 116 amino acid polypeptide called PCT (20). The amino acid sequence of PCT is subsequently cleaved by a convertase enzyme to generate the nPCT of 57 amino acids at the N-terminal, the immature non-amidated calcitonin of 33 amino acids centrally, and the calcitonin carboxyl-terminal peptide-I (CCP-I) of 21 amino acids at the C-terminal (21).

CT-II precursor differs from CT-I precursor only in the last eight amino acids, which produce specific C-terminal peptides (CCP-II). CGRP-I precursor is translated in the N-terminal region into mature CGRP-I, a potent vasodilator that stimulates glomerular filtration, and a cryptic peptide (22). Finally, post-translational amidation produces mature calcitonin, considered to be immunoreactive. Interestingly, thyroid parafollicular cells and neuroendocrine cells in the lungs and bowel produce PCT as well as calcitonin (23). PCT is not secreted into blood, but may be released during bacterial infections and is widely used as a marker in such situations (24). It is also considered a useful biomarker in the diagnosis and follow-up of MTC (25). In healthy individuals all these component peptides, including PCT, are present at very low serum concentrations, and are produced by the neuroendocrine cells in the lungs and thyroid gland (26).

Recent papers have studied the possible relationship between calcitonin or PCT and miRNAs (27, 28, 29, 30). miRNAs are implicated in biological processes such as cellular differentiation, proliferation, and apoptosis; they are stable and can be detected in clinical samples and used as biomarkers for the early diagnosis of several diseases (31). miR-323 is reportedly highly expressed in MTC and some articles support its role in thyroid malignancies (32), but conversely, a recent study found no significant relationship between miR-323 and serum calcitonin levels in MTC patients with or without RET mutation (28). In contrast, there was a statistically significant negative association between miR-224 expression and serum calcitonin levels on diagnosis in a large cohort of MTC patients (27). miR-224 can in fact be considered an independent prognostic marker, given its significant association with patient survival (27). Finally, miR-125b regulates PCT expression by mediating the transcriptional activity of STAT3 in human monocytes (30). miR-125b enhances the production of total STAT3 and phosphorylated STAT3 while reducing the PCT expression of CT (33). A very interesting future research field could be investigation of the correlation between overexpressed miRNAs and PCT or calcitonin levels in MTC and NENs, their biological effects in the pathogenesis of human cancers and their use as prognostic biomarkers in MTC patients.

### Tissue expression of calcitonin

A broad spectrum of NENs expresses calcitonin in their tissue. In the last 30 years it has been established that not only is calcitonin not specific for MTC, but that some MTCs do not express it at all, making their pathologic classification unclear (34). They were initially referred to as ‘atypical medullary thyroid carcinomas’, then as ‘calcitonin-negative neuroendocrine tumors of the thyroid’ or ‘non-medullary neuroendocrine tumors of the thyroid’.

Several cases of extra-thyroid calcitonin-expressing NENs have been described over the years. Prostate cancers with NE dedifferentiation have also been reported. In one of the first studies, 53 prostate cancer cases were analyzed for neuroendocrine dedifferentiation: eight of them were found to express neuroendocrine markers and, of these, two were immunoreactive for calcitonin (35). In a later case series of 42 specimens of radical prostatectomy (36), the presence of calcitonin-positive neuroendocrine cells was demonstrated in normal tissue: levels were lower in hyperplastic nodules of benign prostatic hyperplasia and markedly reduced in cases of prostate cancer. This finding conflicted with those observed in both established prostate cancer cell lines and tissue from human primary tumors (37, 38). These authors not only observed higher calcitonin mRNA expression in cancer compared to normal and hyperplastic tissues, but they also localized its expression within the tissue. It was more common in the basal epithelial cells than in the luminal epithelium and stromal prostate compartment in benign and low-grade cancers, while it was detected throughout the luminal epithelium in higher grade tumors (Gleason score 7–10), and with a stronger signal. Furthermore, tumor progression has been associated with a loss of spatial specificity and increased intensity of staining: cases with higher expression were those that displayed metastatic disease and poor prognosis (39). However,
calcitonin may have a predictive and therapeutic role as well as a potential diagnostic and prognostic role in prostate cancer. Experiments on cell lines revealed that calcitonin expression is associated with chemoresistance to etoposide, dexamethasone and selenite through activation of the Akt-survivin pathway that establishes apoptosis resistance (38).

The second most common site for calcitonin-expressing NENs is the larynx, with more than 80 cases described in the last 30 years. In a case series of 20 laryngectomy specimens (40), non-neoplastic neuroendocrine cells accounted for around 5% of the total number of epithelial cells in laryngeal tissue, mostly located in the middle layer of the respiratory epithelium of the ventricle and subglottic region. The specimens were all reactive to chromogranin A and synaptophysin, but not to calcitonin. Laryngeal NENs are rare (around 0.6% of laryngeal malignancies), but they account for 59% of non-squamous carcinomas arising in this site. They are usually large cell neuroendocrine carcinomas and, like MTC, are often associated with stromal amyloid and TTF1 (albeit focal in laryngeal tumors) and calcitonin immunoreactivity (in 80% of cases) (41). Differential diagnosis between thyroid and laryngeal primary forms is thus based on the integration of clinical, biochemical and radiological data (42). Over 90% of laryngeal NENs arise near the aryepiglottic fold, arytenoid or false vocal cord, while thyroid NENs invade the subglottis or trachea, sparing the supraglottis. Skin metastases are more common in laryngeal neuroendocrine carcinomas than in medullary thyroid carcinomas (40).

Calcitonin expression is common in pancreatic NENs (pNENs) (Fig. 1), but most of our knowledge comes from single case reports where the finding was consequent to the discovery of inappropriately high serum calcitonin levels. A recent study explored the effective frequency of calcitonin tissue expression and assessed the clinicopathological features of this subgroup of pNENs (25 out of 229 cases) (43). More than 10% of them showed various proportions of calcitonin immunostaining, independently of their functional state. In functioning tumors, calcitonin was co-expressed almost exclusively with insulin. A possible explanation is that calcitonin belongs to the same amylin/islet amyloid polypeptide superfamily that is co-secreted with insulin. However, no differences in clinicopathological parameters were seen compared to non-calcitonin expressing tumors. Differential diagnosis between primitive pancreatic MTC and MTC metastasized to the pancreas may be based on the detection of TTF1 immunoreactivity in the latter but not in the former.

The immunohistochemical expression of calcitonin was also assessed in pheochromocytomas (44, 45, 46), an unusual form of NEN. Reactivity confined to cellular groups was observed in 34% of sporadic forms, in 43% of forms occurring in the context of multiple endocrine neoplasia (MEN) syndrome and in 25% of other forms and syndromes (e.g. von Hippel Lindau).

**Calcitonin measurement**

**Methods**

Calcitonin can be detected in the blood using several methods. It was first measured by radioimmuno assay (RIA), using polyclonal antibodies able to recognize mature and immature monomers of calcitonin and other circulating forms (precursors of calcitonin and degradation products) (47). However, this method lacks both specificity and sensitivity, leading to its replacement by two-sided immuno radiometric assays (IRMAS). This improved specificity through the use of two monoclonal antibodies that recognize two different specific epitopes.

**Figure 1**

Histopathological features of a calcitonin-secreting pancreatic NEN (A). A neuroendocrine neoplasm of the pancreas can be seen in the lower part of this field. It was composed of mid-sized cells with nuclei characterized by ‘salt and pepper’ chromatin and granular eosinophilic cytoplasm. (Hemotoxylin and eosin stain, 100× magnification). One year later, this neoplasm caused distant metastasis to the adrenal gland. (B and C) Cells were diffusely immunoreactive to chromogranin (B) and focally to calcitonin (C) staining (100× magnification). d. Ki67 Labeling index was around 5% in a ‘hot spot’ area (400× magnification).
in calcitonin (48). The first antibody, labeled with $^{125}\text{I}$ tracer, binds the calcitonin region in the 11–17 amino acid sequence, while the second binds the solid phase and the amino-terminal (26–32) region. The calcitonin molecules are captured in a sandwich complex, whose radioactivity is directly proportional to the calcitonin content. The limit of detection of this assay is 2.5 pg/mL (48).

However, plasma calcitonin levels are increasingly analyzed by a specific sandwich enzyme-linked immunooassay (ELISA) technique. Since 2000, test manufacturers have moved from radiolabeled systems to fluorescent and chemiluminescent tests, which have been used for MTC identification (49). The chemiluminescence test (ILMA) minimizes cross-reactivity and recognizes the monomeric form of serum calcitonin, enabling the early identification of patients with recurrent MTC (50). ILMA also uses two monoclonal antibodies, but unlike IRMA, they recognize the 11–23 and 21–32 regions and the limit of detection is 1 pg/mL. This makes it more efficient than IRMA, especially for detecting low serum concentrations (49).

In some cases, heterophilic antibodies interfere with calcitonin measurement, leading to a false positive or falsely higher result. Two-site immunometric chemiluminescent assays (ICMAs), which are highly specific for monomeric calcitonin, avoid this problem (51). Despite this, a recent case report found falsely high calcitonin levels with ICMA due to heterophilic antibody interference, and in the same paper a literature review revealed a number of cases of heterophilic antibody interference with ICMA, IRMA and chemiluminescence assay (CLIA) (52). When calcitonin levels are altered due to interference, as in the case of heterophilic antibodies or macrocalcitonin (53), a calcitonin stimulation test (calcium and/or pentagastrin test) or a dilution test (lack of linearity) could be considered.

Recent years have seen the development of numerous automated calcitonin assays, such as those produced by Nichols Institute Laboratories (NID), Diagnostic Product Corporation (DPC) and DiaSorin (DS). Their clinical and analytical quality have been evaluated in many papers, revealing discrepancies in the identification of immunoreactive calcitonin, and important differences in their limits of detection (54). Camacho et al. developed a new method for the measurement of serum calcitonin, an immunofluorometric assay (IFMA) with a limit of detection of 1 pg/mL (55). It is based on two monoclonal antibodies, one linked to the solid phase and specific for the 11–23 amino acid sequence, and the other biotinylated and specific for the 17–32 amino acid sequence (55).

The latest assay developed to measure serum calcitonin is electrochemiluminescence immunoassay (ECLIA). This is a sandwich immunoassay based on streptavidin-biotin technology. One of the calcitonin-specific antibodies is biotinylated and binds to streptavidin-coated microparticles. The second antibody, labeled with a ruthenium complex, is used for calcitonin detection. The automated ECLIA assay benefits from a shorter test time and broader measurement range, thereby enabling the prompt, accurate diagnosis of calcitonin-related diseases (54, 56).

**Interpretation of calcitonin levels**

**Normal calcitonin levels according to age, gender, and lifestyle habits**

Calcitonin levels should be interpreted according to age and gender (57). They are normally higher in the pediatric population. Using a CLIA (Inmulite 2000 XPi, from Siemens Diagnostics), Castagna et al. showed in a group of 2740 pediatric subjects aged up to 16 years that mean serum calcitonin levels were higher during the first and second year of life (9.81 ± 8.8 pg/mL, range 2.0–48.9, and 4.56 ± 2.64 pg/mL, range 2.0–14.7, respectively) compared to older ages (58). The highest levels of all were observed in babies under 6 months (11.3 ± 9.5 pg/mL, range 2.0–48.9). Calcitonin levels dropped significantly from the third year of life, becoming similar to those in adults. No difference between the genders was seen in this population (58). A limitation of this study might be the method, as calcitonin was only detected in 38.5% of the analyzed samples.

In a very recent study, Eckelt et al. used the more sensitive ECLIA (Cobas System, Roche Diagnostics GmbH) to assess calcitonin levels in a cohort of 6090 samples from Caucasian pediatric subjects aged up to 18 years, finding detectable calcitonin levels in 89.5% of samples (59). In accordance with the previous study, the highest levels were observed in newborns aged under 3 months (mean 21.3 ± 10.3 pg/mL, range 6.1–72.7 in boys, and 18.2 ± 6.6 pg/mL, range 8.4–40.0 in girls). It also confirmed that calcitonin levels drop rapidly between 1 month and 4 years for boys and 5 years for girls. In contrast with Castagna et al., Eckelt et al. found that calcitonin levels decreased moderately between 4/5 years and 9.5 years, converging to the reference range for adults starting from 12.5 years for boys and 13 years for girls (59). The authors also found significantly higher calcitonin levels in boys than girls from the second year of life.
Gender differences in calcitonin levels have also been observed in healthy adults (54, 57). A recent prospective study evaluating 783 healthy subjects (398 women and 385 men) by ECLIA showed median calcitonin levels of 0.8 pg/mL (range 0.5–1.8, peak 12.7 pg/mL) in women and 3 pg/mL (range 1.6–5.0, peak 18.0 pg/mL) in men (54). All these studies confirmed the recommendation in the American Thyroid Association (ATA) guidelines that serum calcitonin concentrations should be interpreted in the setting of gender-specific reference intervals (60). This difference is because men have twice as many C cells as women (61).

Lifestyle habits may be linked to moderate hypercalcitoninemia. In men, smoking was associated with significantly higher calcitonin levels (>10 pg/mL, peak 21.4 pg/mL) compared to non-smokers (57). Female smokers also showed higher calcitonin levels than non-smokers, although the difference was not significant. Alcohol consumption also seems to affect calcitonin concentrations. Acute alcohol ingestion results in increased plasma calcitonin levels (62), whereas contrasting results are reported for chronic alcohol use (63, 64). However, physical activity does not seem to have a noticeable effect on basal calcitonin concentration (65, 66).

### False positives and false negatives

Elevated serum calcitonin levels are highly sensitive for MTC, but they are not highly specific (Table 1). Several drugs can stimulate calcitonin secretion (Table 1): for example, proton pump inhibitors (PPI) induce calcitonin secretion after 2-4 months of treatment, through an increase in gastrin hypersecretion (67, 68). Furthermore, several thyroid and non-thyroid diseases may be associated with low-moderate increases in serum calcitonin concentrations. Up to 49% of C-cell hyperplasia (CCH) cases are associated with slightly elevated basal (between 10 and 20 pg/mL) and stimulated (<560 pg/mL) calcitonin levels (54, 57, 69). Follicular and papillary thyroid carcinomas may also be associated with CCH and slightly high calcitonin levels (70, 71). Cases have also been reported of benign multinodular goiter with false positive calcitonin associated with neither CCH nor MTC in surgical specimens (72, 73). Discordant data are reported for chronic autoimmune thyroiditis (74, 75, 76). However, case series have reported moderately high calcitonin levels in patient subgroups, probably caused by progressive C-cell damage due to lymphocytic infiltration (76, 77) or, in 20% of patients in one case series, by CCH (61).

Among non-thyroid non-tumor diseases (Table 1), all conditions causing hypergastrinemia (such as atrophic gastritis and gastrinoma) or hypercalcemia (such as hyperparathyroidism) are strongly associated with high serum calcitonin levels (2, 68). Hypergastrinemia and hypercalcemia are, in fact, strong calcitonin secretagogues, and both are used in stimulatory tests to assess calcitonin secretion. In contrast, elevated calcitonin levels in patients with chronic renal insufficiency are related to lower calcitonin clearance (78).

Several types of tumors, including breast cancer and NENs, may also be associated with ectopic calcitonin secretion (2) (Table 1). Hypersecretion of calcitonin by NENs is rare; in fact, in functional NENs, calcitonin secretion is less common than other hormone secretions causing endocrine paraneoplastic syndromes (79). Table 2 summarizes the main features of the calcitonin-secreting NENs reported in the literature (1, 5, 7, 40, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94). Pancreatic, laryngeal and lung NENs are most frequently associated with hypercalcitoninemia, but calcitonin secretion has also been described in duodenal, esophageal, cutaneous and paranasal NENs. The signs and symptoms of calcitonin-secreting NENs are usually due to mass effect, although calcitonin can cause diarrhea in some patients

### Table 1 Factors associated with low-moderately elevated serum calcitonin levels in non-medullary thyroid carcinoma.

<table>
<thead>
<tr>
<th>Factors</th>
<th>Description</th>
</tr>
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<tbody>
<tr>
<td>Drugs</td>
<td>PPI, glucocorticoids, beta-blockers, glucagon, enteroglucagon, pancreozymin</td>
</tr>
<tr>
<td>Heterophilic antibodies</td>
<td>CGRP inhibitors, used for migraine treatment</td>
</tr>
<tr>
<td>Non-thyroid non-tumor diseases</td>
<td>Hypergastrinemia (chronic antrhopathic gastritis, Zollinger-Elisson syndrome)</td>
</tr>
<tr>
<td></td>
<td>Hypercalcemia (including hyperparathyroidism)</td>
</tr>
<tr>
<td></td>
<td>Pseudohypoparathyroidism</td>
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<tr>
<td></td>
<td>Chronic renal insufficiency</td>
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<td></td>
<td>Pernicious anemia</td>
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<td></td>
<td>Hepatic cirrhosis</td>
</tr>
<tr>
<td></td>
<td>Pancreatitis</td>
</tr>
<tr>
<td></td>
<td>Inflammatory states (including sepsis)</td>
</tr>
<tr>
<td>Non-thyroid neoplasms</td>
<td>NENs (including pheochromocytoma, paraganglioma, entero-pancreatic NEN, insulinoma, esophageal NEN, small cell lung carcinoma)</td>
</tr>
<tr>
<td>Breast cancer</td>
<td></td>
</tr>
<tr>
<td>Methodology</td>
<td></td>
</tr>
<tr>
<td>Heterophilic antibodies</td>
<td></td>
</tr>
<tr>
<td>Macrocalcitonin</td>
<td></td>
</tr>
</tbody>
</table>

CGRP, calcitonin gene-related peptide; NENs, neuroendocrine neoplasms; PPI, proton pump inhibitors.
Table 2  Calcitonin secreting NENs reported in the literature.

<table>
<thead>
<tr>
<th>Reference</th>
<th>Cases (n)</th>
<th>Organ-histology</th>
<th>Age</th>
<th>Sex</th>
<th>Symptoms</th>
<th>Basal calcitonin</th>
<th>Treatment</th>
<th>Post-treatment CT</th>
<th>Outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>(42)</td>
<td>1</td>
<td>Larynx – moderately differentiated NET</td>
<td>59</td>
<td>M</td>
<td>Neck mass, painful s.c. nodules</td>
<td>Basal: 50 pg/mL (56.6)</td>
<td>Surgery: octreotide: 30 mg/4 weeks; everolimus: 10 mg/day</td>
<td>N/A</td>
<td>Partial response after 15 months follow-up</td>
</tr>
<tr>
<td>(93)</td>
<td>1</td>
<td>Pancreas – well differentiated NET (metastatic)</td>
<td>59</td>
<td>M</td>
<td>Chronic diarrhea, weight loss of 4 kg</td>
<td>Basal: &gt;2000 pg/mL</td>
<td>Octreotide: 30 mg/4 weeks (increased to 2/weeks); everolimus: 10 mg/day; radioembolization; ondansetron</td>
<td>NA</td>
<td>Persistently elevated Stable disease after 5 years follow-up</td>
</tr>
<tr>
<td>(81)</td>
<td>1</td>
<td>Esophagus – well differentiated NET (not metastatic)</td>
<td>41</td>
<td>F</td>
<td>Mild dysphagia, feeling of food getting stuck</td>
<td>Basal: 570 pg/mL</td>
<td>Endoscopic resection and cervical lymph node dissection</td>
<td>Normalized</td>
<td>Free of disease after 2 years follow up</td>
</tr>
<tr>
<td>(5)</td>
<td>2</td>
<td>Pancreas – well differentiated NET (non-metastatic and metastatic)</td>
<td>53</td>
<td>M</td>
<td>Vague abdominal discomfort</td>
<td>Basal: 253 pg/mL</td>
<td>Distal pancreatectomy</td>
<td>Normalized</td>
<td>Free of disease after 2 years follow up</td>
</tr>
<tr>
<td>(1)</td>
<td>21</td>
<td>9 lung; 10 pancreas; 1 stomach; 1 appendix – 10 well differentiated and 2 poorly differentiated (all metastatic)</td>
<td>52</td>
<td>M: 12 F: 9</td>
<td>8/24 diarrhea (6 also with carcinoid syndrome)</td>
<td>Basal: &gt;100 pg/mL (higher in poorly differentiated and in high grade tumors)</td>
<td>14 resections of primary tumors + median of 2 (1–6) lines of treatment</td>
<td>NA</td>
<td>Metastasis appearance after 6 months (calcitonin increased to 320 pg/mL)</td>
</tr>
<tr>
<td>(7)</td>
<td>1</td>
<td>Larynx – poorly differentiated adenocarcinoma with neuroendocrine features</td>
<td>57</td>
<td>M</td>
<td>Painful neck mass, otalgia, odynophagia, and hoarseness</td>
<td>Basal: 157 pg/mL</td>
<td>Total laryngectomy, bilateral neck dissection + RT</td>
<td>35 pg/mL</td>
<td>Metastasis appearance after 6 months (calcitonin increased to 320 pg/mL)</td>
</tr>
<tr>
<td>(86)</td>
<td>1</td>
<td>Pancreas – well differentiated NET (metastatic to liver)</td>
<td>38</td>
<td>M</td>
<td>Unintentional weight loss, sweating and fatigue</td>
<td>Basal: 1137 pg/mL</td>
<td>Octreotide: 20 mg/4 weeks + 2 cycles of PRRT (yttrium-90-DOTATOC)</td>
<td>After SSA: 612 pg/ml After PRRT: 336 pg/ml</td>
<td>Stable disease and symptoms free after 7 years follow-up</td>
</tr>
</tbody>
</table>

(Continued)
### Table 2 (Continued)

<table>
<thead>
<tr>
<th>Reference</th>
<th>Cases (n)</th>
<th>Organ-histology</th>
<th>Age</th>
<th>Sex</th>
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<tbody>
<tr>
<td>(80)</td>
<td>1</td>
<td>Lung – large cell neuroendocrine carcinoma</td>
<td>47</td>
<td>F</td>
<td>Weight loss, abdominal pain</td>
<td>Baseline 303 pg/mL</td>
<td>CHT (cisplatin etoposide) + 1 cycle PRRT</td>
<td>After CHT: 152.3 pg/mL</td>
<td>PR after CHT; died of pneumonia after 1 cycle PRRT</td>
</tr>
<tr>
<td>(94)</td>
<td>1</td>
<td>Duodenum – well differentiated NET</td>
<td>63</td>
<td>F</td>
<td>Asymptomatic</td>
<td>Baseline 114 pg/mL</td>
<td>Surgery</td>
<td>NA</td>
<td>Normalized</td>
</tr>
<tr>
<td>(90)</td>
<td>2</td>
<td>Pancreas – histology NA</td>
<td>70</td>
<td>F</td>
<td>Final stage: cachexia, general weakness, Abdominal pain</td>
<td>Baseline 625 pg/mL (no increase after PG test)</td>
<td>Patient refused treatment</td>
<td>NA</td>
<td>DOD</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Pancreas – NET</td>
<td>69</td>
<td>F</td>
<td></td>
<td>Baseline 103 (no increase after PG test)</td>
<td>Octreotide: 20 mg/4 weeks for 2 months; left-pancreatectomy, splenectomy, cholecystectomy, atypical liver-resection of one metastasis</td>
<td>86.9 pg/mL (not radically resected tumors)</td>
<td>NA</td>
</tr>
<tr>
<td>(40)</td>
<td>1</td>
<td>Larynx – moderately differentiated NEC</td>
<td>57</td>
<td>M</td>
<td>Hoarseness, dysphagia, voice change, foreign-body sensation in the throat</td>
<td>Elevated (after metastasis: 599 pg/mL)</td>
<td>Laryngectomy and neck dissection</td>
<td>NA</td>
<td>After 27 months: DOD</td>
</tr>
<tr>
<td>(88)</td>
<td>5</td>
<td>1 mediastinal tumor of undetermined origin – poorly differentiated</td>
<td>45</td>
<td>F</td>
<td>NA</td>
<td>Baseline 54.0 pg/mL (55.7)</td>
<td>NA</td>
<td>NA</td>
<td>After 7 months: DOD</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1 larynx – moderately differentiated large cell NEC</td>
<td>61</td>
<td>M</td>
<td>NA</td>
<td>Baseline 16.3 mg/mL (29.2)</td>
<td>NA</td>
<td>NA</td>
<td>After 32 months: DOD</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3 lung – small cell NEC</td>
<td>66</td>
<td>M</td>
<td>NA</td>
<td>Baseline 585 mg/mL (651)</td>
<td>NA</td>
<td>NA</td>
<td>After 22 months: DOD</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>71</td>
<td>M</td>
<td>NA</td>
<td>Baseline 44.7 mg/mL (49.1)</td>
<td>NA</td>
<td>NA</td>
<td>After 21 months: DOD</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>71</td>
<td>M</td>
<td>NA</td>
<td>Baseline 42.9 mg/mL (43.4)</td>
<td>NA</td>
<td>NA</td>
<td>After 1 month: DOD</td>
</tr>
<tr>
<td>(87)</td>
<td>1</td>
<td>Lung – well differentiated NET</td>
<td>58</td>
<td>M</td>
<td>NA</td>
<td>Baseline 730 pg/mL (758)</td>
<td>Surgery + RT (for primary tumor); CHT for subsequent thyroid metastasis</td>
<td>Normalization after CHT</td>
<td>Free of disease</td>
</tr>
<tr>
<td>(83)</td>
<td>1</td>
<td>Larynx – moderately differentiated NEC</td>
<td>69</td>
<td>M</td>
<td>Hoarseness</td>
<td>Baseline 970 pg/mL</td>
<td>Partial laryngectomy + RT; surgery for subsequent local recurrence</td>
<td>45 pg/mL (rising to 1210 pg/mL after recurrence)</td>
<td>Free of disease</td>
</tr>
</tbody>
</table>
Calcitonin in NENs

Basal and stimulated CT: the importance of gender-specific cut-offs

The routine measurement of serum calcitonin is controversial, as there are various doubts over its real advantage in the consistent detection of MTC. The ATA guidelines on MTC (60) and thyroid nodules (82) do not offer any definitive recommendations for or against calcitonin measurement in the diagnostic work-up of thyroid nodules. Conversely, several European authors support its use for the early diagnosis of MTC, enabling curative surgery and improved survival (99, 100). Although calcitonin values higher than 100 pg/mL are strongly suspicious of malignancy (60), MTCs may also be diagnosed in patients with lower values.

To improve the diagnostic performance of calcitonin assay, a calcitonin stimulation test may be applied in patients with moderately elevated values. However, there is no definitive basal or stimulated calcitonin cut-off values that consistently and accurately discriminate between MTC and other conditions associated with elevated calcitonin levels. The reasons for this lie in various unsolved questions, mainly in relation to differences in inter-laboratory and inter-assay calcitonin measurements. Indeed, current guidelines (60) recommend each center adopts its own cut-off value for basal and stimulated calcitonin.

Several studies describe the prevalence of MTC in patients with thyroid nodular disease and elevated basal calcitonin values. Although they did not all adopt the same cut-off value to identify patients with possible MTC, most reported that more than 40% of patients with basal calcitonin above 10–30 pg/mL had MTC (99, 101, 102, 103, 104, 105, 106). However, due to the retrospective design of the studies and the heterogeneity of the sample
populations, significantly lower (from 3.6 to 22.7%) (77, 107, 108) or even higher (94.7%) rates of MTC were reported by other authors (103) adopting the same cut-off values.

To improve diagnostic performance, it may be helpful to evaluate calcitonin values after stimulation with pentagastrin or calcium. Given the limited availability of pentagastrin in recent years, calcium stimulation may be a valid alternative to predict the presence of MTC in patients with elevated basal calcitonin. As noted above, there is no unique basal calcitonin threshold predictive of the presence of MTC, and stimulated calcitonin may also fail to correctly diagnose MTC, especially when it leads to only mildly increased calcitonin levels. In fact, the stimulated calcitonin values observed in patients with MTC may also be reached in patients with CCH (109).

For instance, pentagastrin-stimulated calcitonin values between 10 and 205 pg/mL have been found with both MTC and CCH (102).

In healthy individuals with no history of thyroid disease, calcium administration led to calcitonin elevation up to 119 and 85.7 pg/mL in males and females, respectively (110). Post-operative findings of MTC ranged from 100% to as low as 26.3% in patients indicated for thyroidectomy due to a pentagastrin-stimulated calcitonin level above 100 pg/mL (77, 99, 102, 103, 106, 107, 108, 109, 111, 112). For this reason, the fold increase after stimulation is more reliable than the absolute value of stimulated calcitonin, since stimulated calcitonin values are at least three to four times higher than the basal values in patients with MTC (97).

**Stimulated calcitonin in thyroid NENs (MTC) the calcium stimulation test**

The calcium stimulation test has some noteworthy features. It promotes calcitonin increase to a greater extent than pentagastrin, the peak is achieved 2 min after calcium injection, and males have higher calcium-stimulated calcitonin levels than females (54, 101, 104, 113, 114). Table 3 summarizes the main characteristics of the published studies on calcium-stimulated calcitonin cut-offs for differentiating MTC from non-MTC (including CCH), or MTC from normal tissue, in comparison with the diagnostic accuracy of basal calcitonin. Colombo et al. identified basal calcitonin >18.7 and >68 pg/mL and stimulated calcitonin >184 and >1620 pg/mL in females and males respectively as the best levels to distinguish MTC from non-MTC patients (101). In this study there was no difference in the diagnostic power of pentagastrin and calcium stimulation (101). Furthermore, on C-cell immunohistochemical examination, a stimulated calcitonin value <50 pg/mL corresponded to a mean of 30 cells per 10 fields, whereas a higher stimulated value was associated with a mean of 400 cells per 10 fields, often displaying a diffuse and nodular distribution pattern (101).

Mian et al. found that the best calcium thresholds for the identification of MTC were >26 and >68 pg/mL for basal calcitonin and >79 and >544 pg/mL for stimulated calcitonin in females and males, respectively, with similar diagnostic accuracy (104). Papadakis et al. identified different cut-off values, finding calcium-stimulated calcitonin >452 pg/mL in 75% of males with MTC and >274 pg/mL in 100% of females with MTC (115). Surprisingly, an appreciable number of patients with stimulated calcitonin levels >100 pg/mL presented differentiated thyroid carcinoma of follicular origin (115).

Rosario et al. did not find MTC in any patient with basal calcitonin ≤24.6 pg/mL or stimulated calcitonin ≤186.5 pg/mL, and all patients without MTC had basal calcitonin <47 pg/mL and stimulated calcitonin <655.2 pg/mL. MTC was found in 25% of patients with basal calcitonin between 24.6 and 47 pg/mL (116). The authors also applied the cut-offs proposed by Mian et al., obtaining 83.3% sensitivity and 62.8% specificity, and by Papadakis et al., obtaining 67% sensitivity and 80% specificity (116). There was no agreement on the best cut-off values between series, and none offered both sensitivity and specificity ≥85% (116).

Niederle et al. recently found MTC in all females and males with either basal calcitonin >23 and >43 pg/mL or calcium -stimulated calcitonin >780 and >1500 pg/mL, respectively (114). In this study early-peak stimulated calcitonin was found in CCH or cases with a low tumor burden (114). Pentagastrin stimulation was also used, with a similar diagnostic power (114). The same group confirmed these basal and calcium -stimulated calcitonin cut-offs in a recent publication (117), and showed that combining the two values (basal plus stimulated) did not improve diagnostic accuracy (117). They also found that a basal calcitonin cut-off of >85 pg/mL for females and >100 pg/mL for males showed 100% sensitivity for diagnosing lateral neck lymph node metastasis.

Unfortunately, the available studies on calcitonin values after calcium administration adopted different calcium infusion protocols, with elemental calcium doses ranging from 2.3 to 2.5 mg/kg and i.v. infusion times from 30 s to 5–10 mL/min. Furthermore, the timing of the blood samples was not standardized (taken at various times
### Table 3
Cut-offs of basal and calcium-stimulated CT.

<table>
<thead>
<tr>
<th>Reference</th>
<th>Study population</th>
<th>Aim</th>
<th>Cut-off bCT (pg/mL)</th>
<th>Se (%)</th>
<th>Sp (%)</th>
<th>PPV (%)</th>
<th>NPV (%)</th>
<th>Ca stimulation test: dose and infusion</th>
<th>Timing of blood samples</th>
<th>Cut-off sCT (pg/mL)</th>
<th>Se (%)</th>
<th>Sp (%)</th>
<th>PPV (%)</th>
<th>NPV (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>(90)</td>
<td>40 pts with nodular goiter</td>
<td>1. Differentiating MTC from non-MTC</td>
<td>M: 68</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>2.3 mg/kg of elemental Ca infused at 10 mL/min</td>
<td>0, 2, 5 and 15 min</td>
<td>M: 1620</td>
<td>75</td>
<td>100</td>
<td>100</td>
<td>99.9</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2. Differentiating MTC+CCH vs normal</td>
<td>F: 18.7</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>2.3 mg/kg of elemental Ca infused at 10 mL/min</td>
<td>0, 2, 5 and 15 min</td>
<td>F: 184</td>
<td>100</td>
<td>92.9</td>
<td>6.6</td>
<td>100</td>
</tr>
<tr>
<td>(93)</td>
<td>91 pts with thyroid nodules</td>
<td>1. Differentiating MTC from non-MTC</td>
<td>M: 68</td>
<td>83.3</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>2.3 mg/kg of elemental Ca infused at 10 mL/min</td>
<td>0, 2, 5 and 10 min</td>
<td>M: 32.6</td>
<td>100</td>
<td>85.4</td>
<td>68.4</td>
<td>89.2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2. Differentiating MTC+CCH from normal</td>
<td>F: 26</td>
<td>81.8</td>
<td>97.9</td>
<td>68.6</td>
<td>76.9</td>
<td>2.3 mg/kg of elemental Ca infused at 5 mL/min, min. time of 3 min</td>
<td>0, 2, 5 and 10 min</td>
<td>F: 79</td>
<td>100</td>
<td>93.8</td>
<td>71</td>
<td>64.7</td>
</tr>
<tr>
<td>(104)</td>
<td>54 pts with thyroid nodules and sCT &gt;100</td>
<td>Differentiating MTC from CCH</td>
<td>F: 10</td>
<td>75</td>
<td>71</td>
<td>88.5</td>
<td>67</td>
<td>2.3 mg/kg of elemental Ca infused in 3 min</td>
<td>0, 1, 2, 3 and 5 min</td>
<td>F: 55</td>
<td>85</td>
<td>86</td>
<td>59.6</td>
<td>79.89</td>
</tr>
<tr>
<td></td>
<td></td>
<td>M: 17.4</td>
<td>91.9</td>
<td>80</td>
<td>63</td>
<td>55</td>
<td>85</td>
<td>2.3 mg/kg of elemental Ca infused in 3 min</td>
<td>0, 1, 2, 3 and 5 min</td>
<td>M: 452</td>
<td>75</td>
<td>80</td>
<td>75</td>
<td>100</td>
</tr>
<tr>
<td>(105)</td>
<td>41 pts with thyroid nodules and 10 &lt; bCT 100</td>
<td>Differentiating MTC from non-MTC</td>
<td>F: 10</td>
<td>50</td>
<td>100</td>
<td>100</td>
<td>92.1</td>
<td>2.5 mg/kg of elemental Ca infused at 10 mL/min</td>
<td>0, 2, 5 and 10 min</td>
<td>M: 186*</td>
<td>185</td>
<td>60</td>
<td>30</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td></td>
<td>M: 24.6*</td>
<td>74.3</td>
<td>40</td>
<td>100</td>
<td>100</td>
<td>92.1</td>
<td>2.5 mg/kg of elemental Ca infused at 10 mL/min</td>
<td>0, 2, 5 and 10 min</td>
<td>F: 655.2*</td>
<td>33.3</td>
<td>100</td>
<td>100</td>
<td>98.7</td>
</tr>
<tr>
<td></td>
<td></td>
<td>F: 655.2*</td>
<td>83.3</td>
<td>62.8</td>
<td>27.7</td>
<td>95.6</td>
<td>186*</td>
<td>60</td>
<td>30</td>
<td>100</td>
<td>98.7</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>M: 544*</td>
<td>79°</td>
<td>79°</td>
<td>79°</td>
<td>79°</td>
<td>186*</td>
<td>60</td>
<td>30</td>
<td>100</td>
<td>98.7</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>F: 274°</td>
<td>66.6</td>
<td>80</td>
<td>36.3</td>
<td>93.3</td>
<td>186*</td>
<td>60</td>
<td>30</td>
<td>100</td>
<td>98.7</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(106)</td>
<td>149 consecutive pts with thyroid nodules</td>
<td>1. Differentiating MTC from non-MTC</td>
<td>M: 43</td>
<td>53</td>
<td>100</td>
<td>100</td>
<td>67</td>
<td>2.5 mg/kg of elemental Ca infused in 30 s</td>
<td>0, 2, 3 and 5 min</td>
<td>M: 1500</td>
<td>48</td>
<td>100</td>
<td>100</td>
<td>65.76</td>
</tr>
</tbody>
</table>

(Continued)
Calcitonin in NENs

Calcitonin in extra-thyroid NENs

Elevated calcitonin may also be caused by extra-thyroid NENs (Table 2). In a multicenter prospective study for the analytical performance and clinical validation of basal serum calcitonin concentrations, more than 10% of NENs had serum calcitonin concentrations above the 97.5th percentile (54). For basal calcitonin in the gray area, a confirmatory stimulation test should help in differential diagnosis with MTC (104), although no specific cut-offs for stimulated calcitonin in this setting have been suggested in the literature. In fact, only a handful of case reports and small case series have reported calcitonin values after pentagastrin stimulation in calcitonin-secreting NENs (Table 2). The first published case was of a man with a laryngeal NEN that showed a poor response to pentagastrin, with basal calcitonin 1200 ng/L (the authors reported <200 ng/L as normal) and stimulated calcitonin 1500 and 1100 ng/L at 3 and 5 min, respectively, after pentagastrin administration (92). In contrast, in a second case, again involving a man with a laryngeal NEN, calcitonin levels doubled from 3790 pg/mL at baseline (normal value <10 pg/mL) to 6378 pg/mL 5 min after pentagastrin stimulation (91). In a prospective study on the suitability of pentagastrin stimulation in differential diagnosis of NENs of the foregut, Machens et al. found that a less than two-fold increase in serum calcitonin levels was significantly correlated with both non-MTC NENs (one mediastinal and one laryngeal NEN) and small cell lung carcinoma (SCLC) (3 cases) (88).

Immunostaining of tissue specimens for calcitonin was
positive in the patients with mediastinal and laryngeal NEN, but negative in the two SCLC patients with adequate tissue samples. Similarly, another case of lung NET showed no increase in calcitonin levels after pentagastrin (730 pg/mL at baseline, 758 pg/mL after stimulation) (87), nor did two cases of women with pNENs (90).

The unavailability of pentagastrin in most countries has led to the need to standardize other stimulation tests, including for the differentiation of calcitonin-secreting NENs from MTC. In a recent case report (42) calcium-stimulated calcitonin was included in the diagnostic work-up of a calcitonin-secreting laryngeal NEN. The basal calcitonin was 50 pg/mL (normal value <10 pg/mL), increasing to a peak of 56.6 pg/mL 10 min after calcium infusion. The calcium test was considered negative because of the low calcitonin peak value, even though the baseline value was five times higher than the normal range. This result, together with the negative measurement of calcitonin in the fine-needle aspirate washout (FNA-CT), suggested that the calcitonin production was extra-thyroid in origin; this was confirmed by the histological thyroid examination.

The measurement of basal and stimulated calcitonin should therefore be included in the differential diagnosis between thyroid and extra-thyroid NENs, but further studies are needed to define the specific cut-offs.

**Calcitonin measurement in fine-needle aspirate washout**

Ultrasound-guided fine-needle aspiration cytology (FNAC) of a thyroid nodule is often used for the preoperative diagnosis of MTC, although its diagnostic accuracy is not as high as for papillary thyroid carcinoma (PTC), because of the low cellularity, variety of cellular morphologies, and non-typical cell shapes that could be present in MTC (119, 120, 121). The reported accuracy of FNAC in diagnosing MTC in patients with nodules ranges from 50 to 85% (120, 121, 122, 123). Several studies found that the FNA-CT from thyroid nodules may significantly improve the sensitivity of MTC diagnosis (123, 124, 125, 126, 127). De Crea et al. used CLIA (Liaison XL, DiaSorin) to measure calcitonin, finding that the highest sensitivity and accuracy for MTC diagnosis by FNA-CT was achieved with cut-off values of >10.4 pg/mL and FNA-CT/serum calcitonin ratio >1.39. The authors showed that the FNA-CT/serum calcitonin ratio was particularly useful in patients with extremely high serum calcitonin levels, as these are potentially caused by peripheral blood contamination of needle washout fluid, with improved accuracy over both FNA-CT alone (9% vs 85%) and FNAC alone (90 vs 85%) (123). A more recent study measured FNA-CT levels by ECLIA with the Elecsys calcitonin test system (Roche Diagnostics), reporting a cut-off value for FNA-CT of 21.0 pg/mL, leading to 100% sensitivity and 100% specificity in distinguishing MTC-nodules from non-MTC nodules (127).

The diagnostic accuracy of FNA analysis has been reported to be markedly increased by immunocytochemistry for calcitonin in the FNA specimen, particularly when cytological morphology is ‘unusual’ in the presence of elevated serum calcitonin levels. However, this procedure is limited by technical concerns about the great pre-analytical variability and the need for samples with adequate cellularity, and it is not specific for MTC (CCH is a false positive) (124). Based on this evidence, the ATA guidelines recommend that when FNA findings are inconclusive or suggestive of MTC, calcitonin should be measured in the FNA washout fluid and immunocytochemical staining should be performed in the FNA sample to detect the presence of markers such as calcitonin, chromogranin and CEA and the absence of thyroglobulin (Grade B Recommendation) (60).

**Clinical significance**

As reported in a recent systematic review on 72 368 patients from 16 studies, only 0.32% of patients with thyroid nodules were diagnosed with MTC (128). Given this low prevalence, even though the sensitivity and specificity of basal and stimulated CT are very high, the need for routine CT measurement in patients with thyroid nodules is still under debate (128).

Based on the available evidence, we propose a diagnostic algorithm for patients with thyroid nodules and high calcitonin serum levels, reported in Fig. 2. After excluding any interferences, we recommend confirming high calcitonin levels by repeating measurements. If calcitonin levels normalize after eliminating confounding factors, diagnostic procedures may be stopped; otherwise, further examinations depend on their levels. For calcitonin >100 pg/mL, thyroid ultrasound is mandatory to identify suspicious thyroid nodules, which require subsequent FNAC and CT-FNA, while immunocytochemistry for calcitonin is considered optional. For borderline values (10–100 pg/mL), a calcium stimulation test could be helpful. Although cut-off values vary in different studies, the result can be assessed in relation to the increase from the baseline. A stimulated calcitonin value less than twice...
the baseline is not suggestive of MTC and supports the execution of whole-body imaging. A value of three-four times the baseline, reaching a peak >80 pg/mL in women and >100 pg/mL in men, is very suggestive of MTC and therefore requires FNAC and CT-FNA.

In our opinion, like that of other authors (76, 99, 107, 111), CT serum testing in the context of thyroid nodule evaluation enables the early diagnosis of a potentially more aggressive tumor and improves sensitivity of the FNAC, which actually confirms the diagnosis of MTC in less than 50% of patients (120). Our opinion is supported by the fact that the high reliability and sensitivity of the new immunochemiluminometric CT measurement completely eliminates cross-reactivity with procalcitonin and other related peptides, which leads to falsely elevated serum CT levels (heterophilic antibodies and macrocalcitonin) in up to 3.7% of cases (57).

Conclusions

The interpretation of increased serum calcitonin is still a gray area in endocrinology given the many confounding factors, the multiple assays and protocols for stimulation tests, the different cut-offs for both basal and stimulated calcitonin, and above all, its possible secretion by both non-neuroendocrine and neuroendocrine neoplasms. While a higher calcitonin serum level suggests MTC, it is not a pathognomonic sign. A careful diagnostic evaluation of all patients presenting with hypercalcitoninemia is therefore essential for an accurate early diagnosis identifying the thyroidal or extra-thyroidal origin of the secretion, essential for its correct management.

Declarations of interest

Andrea Isidori is an Associate Editor of European Journal of Endocrinology. He was not involved in the editorial or peer review process for this paper, on which he is listed as an author. The other authors have nothing to disclose.

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Author contribution statement

E Gi, V G, B A, E Gu, P M conceived and designed the review. E Gi, V G, B A, P M, C S, T F, G P collected the data and co-wrote the manuscript. E Gu conceived the tissue expression section and Fig. 1. A M I, A A L C and A F contributed to the revision of the manuscript. All authors read and approved the final manuscript.

https://eje.bioscientifica.com
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Calcitonin in NENs


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Calcitonin is a hormone produced by the C cells of the thyroid gland. It plays a role in calcium homeostasis and has been implicated in the development of medullary thyroid carcinoma (MTC), a rare but aggressive form of thyroid cancer. The diagnosis of MTC often involves measuring calcitonin levels in the serum. This hormone is also associated with other conditions, such as medullary thyroid cancer (MTC) and non-adenomatous, non-endocrine thyroid neoplasms.

**Calcitonin in NEs:**

Calcitonin is secreted by the parafollicular C cells of the thyroid gland. Its primary role is in the regulation of plasma calcium levels. It is also involved in the development of medullary thyroid carcinoma (MTC), a rare but aggressive form of thyroid cancer. The diagnosis of MTC often involves measuring calcitonin levels in the serum. This hormone is also associated with other conditions, such as medullary thyroid cancer (MTC) and non-adenomatous, non-endocrine thyroid neoplasms.

**Calcitonin-negative MTC:**

Calcitonin-negative medullary thyroid carcinoma (MTC) is a rare and challenging diagnosis. It can be difficult to distinguish from other thyroid conditions, such as nodular goiter, and it may be associated with other endocrine neoplasms. The diagnosis of calcitonin-negative MTC is often made based on the results of imaging studies and the absence of calcitonin secretion.

**Calcitonin in sporadic MTC:**

Sporadic medullary thyroid carcinoma (MTC) is a rare form of thyroid cancer that affects people with no family history of the disease. It is often diagnosed at an advanced stage due to its asymptomatic nature. The diagnosis of sporadic MTC is often made based on the results of imaging studies and the absence of calcitonin secretion.

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