Medullary Thyroid Cancer Diagnosis: An Appraisal

Pierpaolo Trimboli 1, Luca Giovanella 2, Anna Crescenzi 3,4, Francesco Romanelli 5, Stefano Valabrega 6, Giuseppe Spriano 7, Nadia Cremonini 8, Rinaldo Guglielmi 9, Enrico Papini 9

1 Section of Endocrinology and Diabetology, Ospedale Israelitico, Rome, Italy.
2 Department of Nuclear Medicine and Thyroid Centre, Oncology Institute of Southern Switzerland, Bellinzona, Switzerland.
3 Section of Pathology, Ospedale Israelitico, Rome, Italy.
4 Anatomic Pathology Unit, Ospedale Regina Apostolorum, Albano Laziale, Rome, Italy.
5 Department of Experimental Medicine, Sapienza University, Rome, Italy.
6 Department of Medical and Surgical Sciences, Ospedale S. Andrea, Sapienza University, Rome, Italy.
7 Department of Otolaryngology, Head & Neck Surgery, Istituto Nazionale Tumori Regina Elena, Rome, Italy.
8 Endocrinology Unit, Ospedale Maggiore, Bologna, Italy.
9 Department of Endocrinology, Ospedale Regina Apostolorum, Albano Laziale, Rome, Italy.

Corresponding author:
Dr. Enrico Papini
Department of Endocrinology, Ospedale Regina Apostolorum,
Via San Francesco 50, 00041 – Albano (Rome), Italy
Tel: + 39 333 6235608
e-mail: enrico.papini@fastwebnet.it

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Abstract

Since its first description in 1951, a timely diagnosis of medullary thyroid cancer (MTC) may represent a diagnostic challenge in clinical practice. Several contributes have been addressed to the treatment and follow-up of MTC, but review papers focused on the diagnostic problems of this cancer in clinical practice are sparse. As a delayed diagnosis and an inadequate initial treatment may severely affect the prognosis of this thyroid malignancy, the appropriate use and the correct interpretation of the available diagnostic tools for MTC is of crucial importance. The present paper is aimed to provide an easy-to-use guide reviewing the main issues of MTC diagnosis:

1. Basal serum Calcitonin (CT)
2. Stimulated serum CT
3. Additional serum markers for MTC
4. Ultrasonography and other imaging techniques
5. Fine needle aspiration cytology (FNA)
6. CT measurement on FNA washout (FNA-CT)
7. RET (REarranged during Transfection) mutations

Scope of the problem

MTC is a malignant tumor originating from thyroid parafollicular C cells and accounts for less than 5% of thyroid cancers (1,2). MTC is part of an autosomal dominant inherited disorder (MEN 2 A and B and familial MTC) in about 20% of cases and presents as sporadic tumor in the remainder (2,3). The identification of MTC as a neuroendocrine tumor producing CT and the use of CT as an immunohistochemical marker improved the histologic assessment of MTC and has resulted in an increased incidence of this malignancy (4).

Since its first description in 1951 (5), the diagnosis of MTC may represent a diagnostic challenge in clinical practice. Fine needle aspiration biopsy (FNA) of thyroid nodules, currently the most accurate tool for detecting thyroid malignancies, reveals a diagnostic accuracy for this cancer type less consistent than for differentiated thyroid carcinoma (6,7). The sensitivity for MTC of serum calcitonin (CT) has been reported as higher than ultrasound (US) examination and cytology but the actual diagnostic accuracy of this marker and its use as a routine test in clinical practice are still a matter of debate.
(1,8,9). Currently, histologic identification of MTC is quite reliable, but the issues of its early clinical detection and pre-operative confirmation remain in part unsettled. Due to these persistent diagnostic gray zones, a part of MTC is still incidentally discovered after thyroid surgery with the risk of an incomplete therapeutic approach and of a less favourable prognosis (10). A tailored surgical approach, including total thyroidectomy, central neck nodal and, in a few cases, lateral neck nodal dissection, is needed for an appropriate treatment (2) but requires an early pre-surgical diagnosis and a correct clinical staging of MTC.

Several original contributes, review articles and consensus documents (2,11) have been addressed to the treatment and follow-up of MTC but reviews focused on the diagnostic problems of this cancer in clinical care are sparse. As a delayed diagnosis and an inadequate initial treatment severely affect the prognosis of this thyroid malignancy, the present paper will be aimed to the following aspects of MTC diagnosis:

1. Basal serum Calcitonin (CT)
2. Stimulated serum CT
3. Additional serum markers for MTC
4. Ultrasonography and other imaging techniques
5. Fine needle aspiration cytology (FNA)
6. CT measurement on FNA washout (FNA-CT)
7. RET (REarranged during Transfection) mutations

Methods

Articles were obtained by searching in PubMed MEDLINE the following search MeSH: medullary carcinoma OR medullary thyroid cancer OR medullary thyroid carcinoma OR RET OR calcitonin. The limits of the search included “humans”, “randomized controller trials” or “meta-analysis”. Also, UpToDate (www.uptodate.com) was browsed. In addition to these articles, numerous additional relevant articles, book chapters, and other materials were also supplied by the authors.

Diagnostic issues

1. Basal serum Calcitonin (CT)

1.1 Which methods are available for the determination of serum CT?
Serum CT determination has evolved over time, with methods changing from competitive radioimmunoassays (RIAs) to ‘sandwich’ immunoradiometric assays (IRMAs) (12). Due to the variable level of the different products of the CT gene, RIA determinations tend to be (up to 10 times) higher than those of sandwich IRMA analytical methods (13). Recently, immunoassays that incorporate non-radioisotopic enzymatic (IEMA) or luminescent (ICMA) methods have become available on fully-automated platforms. Hence, most laboratories have moved routine CT measurement from manual IRMA methods (14, 15) to automated assay platforms with comparable analytical performances (16).

Due to the poor inter-method and inter-laboratory agreement, reflecting differences in calibration and specificity of antibodies (17), the laboratory report of serum CT measurement should specify the type of CT assay, its characteristics and its reference limits (2).

1.2 How to obtain and handle blood samples for CT measurement?

The blood sample should be drawn from a fasting patient. Due to the low stability of serum CT at room temperature, it is necessary to centrifuge the sample immediately after blood coagulation and to freeze and transport it in ice to the laboratory.

1.3 Which are the limits of serum CT in normal population?

Healthy subjects almost always have a serum CT concentration <10 pg/mL on IRMA determination (18). This threshold level was adopted in several clinical guidelines and is widely used in clinical practice (19). The upper reference limit for males, however, should be set at a higher level than for females. The use of gender-specific CT reference ranges (20-23) is supported by autopic studies which demonstrate that the number of C cells is approximately two times higher in men than in women and by the evidence of a positive relationship between serum CT and thyroid volume (that is higher in males than in females). To establish normal limits of CT in healthy subjects is difficult due to the significant variability in diagnostic accuracy and reference ranges of the different commercially available assays (23).

Each laboratory should assess its own normal references by using data derived from local control series. As an alternative option, the method-specific and gender-specific normal limits derived from relevant literature and manufacturers’ recommendations may be used (Table 1).
1.4 Which are the potential confounding factors in serum CT determination?

Preanalytical factors that may bias CT measurement are blood sampling from a non-fasting subject or the recent intake of alcohol or calcium salts, possible stimulators of CT secretion. Bacterial infections, severe disease status, hypercalcemia (hyperparathyroidism) and renal failure may interfere as well (24). Increased CT may be associated to several non-medullary conditions: autoimmune thyroiditis, pregnancy and lactation, leukemia, systemic mastocytosis, small-cell lung carcinoma, breast or pancreatic cancer (25). Hypergastrinemia and treatment with proton-pump-inhibitors for more than two months may be associated with hypercalcitoninaemia as well (26). Spurious hypercalcitoninemia due to heterophilic antibodies (HAb) interference is also rarely reported (20, 27, 28).

In clinical practice, due to these numerous confounding factors, a basal hypercalcitoninaemia should always be confirmed by a second determination after the research and exclusion of the potential interfering conditions (29). Ruled out these circumstances, the confirmation of an elevated CT level should suggest C-cell hyperplasia (CCH) or MTC as the most probable causes.

1.5 What basal serum CT level may be considered suspicious for MTC in clinical practice?

Basal serum CT levels >100 pg/mL are suspicious for MTC when determined with an IRMA or ICMA method (2,29). Serum CT elevations below this value are of difficult interpretation (2) but their predictive value is improved by gender-specific cut-offs. In a series of 26 histologically proved occult MTC and 74 sporadic CCH, the best PPV (88%) was achieved by the use of a 20 pg/mL and a 80 pg/mL threshold in females and males, respectively (30). In a prospective study, preoperative CT levels from 20 to 100 pg/mL resulted associated with surgical finding of CCH in 50% of cases (31).

Currently, basal CT cut-off levels are imperfect and their diagnostic accuracy should be improved with large controlled studies. A practical approach may be based on the results of CT levels in a multicenter consecutive series of patients with unselected thyroid nodules (8). Serum CT levels from 20 (twice the upper reference limit) to 50 pg/mL and from 50 to100 pg/mL demonstrated a PPV for MTC of 8% and 25%, respectively. It is noteworthy that MTC is associated with normal serum CT levels in rare cases only (32,33).
1.6 When should serum CT be measured?

The issue of serum CT screening for patients with thyroid nodules is partially unsettled due to analytical problems, low prevalence of MTC, cost of routine determination in a large population, and risk of inappropriate surgical treatment after misleading results. The European Thyroid Association-Thyroid Cancer Network (34) and the German Society for Endocrinology (35) recommend the use of serum CT for the screening of MTC in patients with thyroid nodules or nodular goiter. Due to uncertainties about its cost-effectiveness, ATA Thyroid Nodule and Cancer Guidelines recommend neither for nor against the routine CT measurement. (2). For these reasons, the AACE/AME/ETA Thyroid Nodule Guideline recommends the determination of serum CT in specific conditions only: subjects with a family history of MTC or MEN, patients with indeterminate FNA cytology, nodules with US findings suspicious for malignancy and all patients with nodular goiter who are undergoing thyroid surgery. These recommendations limit the costs and hazards of routine CT determination, decrease the risk of delayed diagnosis and prevent an inadequate surgical treatment (29).

2. Stimulated serum CT

2.1 Is the diagnostic accuracy of serum CT increased by stimulation?

The available data suggest that the specificity of CT measurement is improved by CT stimulation test (6). In Europe, a widely used method of CT stimulation was the intravenous administration of 0.5 µg/kg of pentagastrin (PG). Serum CT was measured before the injection, and 2 and 5 minutes thereafter (36). A clear-cut CT rise was observed in MTC patients after the injection of PG, and a PG-stimulated CT >100 pg/mL should be considered suspicious for CCH or MTC (2). The reported PPV of the peak of stimulated CT is unfortunately quite variable (from 25% to 80% in different studies) (37). In large series of unselected patients with thyroid nodules, a PG-stimulated CT >1000 pg/mL showed a 100% PPV for MTC, whereas values between 100 and 1000 pg/mL demonstrated a 20%PPV (8). In patients with stimulated CT values between 100 and 1000 pg/ml, the histologic finding of CCH, a premalignant proliferation or the earliest phase in the evolution of a MTC (38), was more frequent than MTC after surgical resection (37). The latter “false positive” diagnosis seems to involve mainly males, even if wide differences are reported (39,30). Limits to the clinical use of CT stimulation test with PG are its difficult
commercial accessibility, pregnancy, old age (>60 years) and concomitant cardiovascular morbidities.

CT stimulation with the intravenous infusion of calcium gluconate (2.5 mg/Kg of elemental calcium) has been proposed as an alternative method. This diagnostic approach was better tolerated by patients and was at least as effective as PG test in stimulating CT secretion in healthy subjects (40). Stimulated CT values (median and 97.5th percentile peak values) obtained two minutes after infusion in normal subjects are 95.4 and 102 pg/mL in males and 90.2 and 78.5 pg/mL in females, respectively (40,41). Patients with multinodular goiter (and no MTC) have peak CT levels up to 2-fold greater than controls after calcium stimulation. In a series of 34 patients with nodular goiter and basal CT >10 pg/mL, stimulated CT levels >184 pg/mL in females and >1620 pg/mL in males provided an accurate cut-off for the detection MTC (42). Because of the high variability of the results from different papers, more robust data are still needed to provide a reliable diagnostic threshold.

In clinical practice, the predictive value for MTC or CCH of an elevated basal CT is increased by a positive stimulation test with calcium. After a stimulated CT peak >100 pg/mL the surgical option should be considered with the patient, especially if peak values are increasing over time.

2.2 When is a stimulation test required in clinical practice?

The trigger basal CT level that suggests the use of a stimulation test varies widely from study to study (range: 5-30 pg/mL). Basal CT concentrations tend to correlate with the tumor mass and may be only slightly elevated in small size MTC (10). The early identification of “micro-medullary cancers” (<10 mm) in nodular goiters with a stimulation test may significantly decrease the number of MTC patients with extrathyroid spread at presentation (43).

In clinical care, a stimulation test should be offered to patients with nodular goiter and a confirmed basal CT value comprised between the upper (gender- and method-specific) reference limit and 100 pg/mL (Table 1).

2.3 Further CT stimulation tests
Serum levels of CT may be stimulated by means of whisky ingestion (44) or omeprazole treatment (45). Due to the small number of reported patients and their low diagnostic accuracy, these tests should not be used in routine clinical care.

3. Additional serum markers for MTC

3.1. Are there additional serum markers for the diagnosis of MTC?

Cells from MTC may produce substances such as carcinoembryonic antigen (CEA), chromogranin A, CT-related peptide, somatostatin, adrenocorticotropic hormone, amyloid, serotonin and vasoactive intestinal peptide. Most of these molecules are of interest only in case of patients with advanced MTC that present syndromes like facial flushing, diarrhea and Cushing features (1,2). Patients with poorly differentiated and more aggressive MTC frequently show a disproportionately high CEA/CT ratio and a rapid CEA doubling time (46). CEA may be considered as a prognostic marker during MTC follow-up and it should be used as a complementary test in aggressive MTC type. Its baseline determination, however, adds little to the initial diagnostic work-up in patients with thyroid nodules. Procalcitonin (PCT), a CT precursor, was reported as an additional serum marker in the diagnosis and follow-up of MTC (41, 47-50). An elevated PCT level may be associated with non-MTC conditions such as systemic inflammation, infection and sepsis. The role of PCT in detecting MTC deserves further studies, including patients with multinodular goitre and autoimmune disease (41, 47-50).

4. Ultrasonography and other imaging techniques

4.1 Are there ultrasound risk features predictive of MTC?

US examination is the pivotal tool for the stratification of the risk of malignancy in thyroid nodules (29). US features, such as hypoechogeticity of the lesion, presence of irregular margins and microcalcifications, intralesional vascular signal, alone or combined, are indicative for a malignant thyroid nodule and strongly suggest FNA biopsy (29, 51, 52). These recommendations are mainly focused on papillary carcinoma (PTC), due to its high prevalence (at least 80% in different series), while few contributes analyzed the US characteristics associated with the much rarer MTC (53-58). MTC may show various aspects at US examination. Two papers (55, 56) compared MTC and PTC sonographic features, one studied MTC vs benign controls (54), and a last one revised a small series of MTC with no controls (53). More recently, 12 MTC and 39 PTC cases were compared with
a control group of benign nodules (58). On the basis of the about ninety MTC cases reported as a whole in these small studies, traditional US risk factors for PTC seem to be inconsistently associated with MTC. Even if the US patterns are not pathognomonic for MTC, however, the finding of a solid and deeply hypoechoic nodule or the presence of intralesional (especially if coarse) calcifications should suggest the possibility of a MTC and prompt the determination of serum CT.

4.2 Are ultrasound features relevant for MTC prognosis?

As approximately 50 percent of patients have clinically detectable cervical lymph node involvement or extracapsular diffusion at diagnosis (10), a careful sonographic evaluation of the neck is always needed in suspicious MTC subjects. US staging may guide the extent of surgery and help to define the preoperative patient prognosis. A study on 77 MTC demonstrated an indolent behavior and a fairly good prognosis in 23 cancers without US risk features, while 54 MTC with “malignant” appearance at US had a more aggressive course (59).

4.3 Other imaging techniques

Computed tomography (CT) and magnetic resonance (MR) are useful for preoperative staging of MTC patients with serum CT>400 pg/mL or neck ultrasound suspicious for extracapsular spread (60). Positron emission tomography (PET) with $^{18}$F-DOPA and $^{18}$F-FDG may be indicated during follow-up of patients with biochemical recurrence of MTC and negative conventional imaging (61).

5. Fine needle aspiration cytology (FNA)

5.1 When should FNA be performed for the diagnosis of MTC?

Cytologic evaluation is the single most informative test for the diagnosis of thyroid malignancy (2,29) and it should always be performed when a MTC is suspected. MTC cytology is characterized by high cellularity with single cells or small clusters, absent colloid and a variable amount of amiloid substance (positive at Congo-red staining) homogeneous, in rods or spheres (38). Cytomorphology consists predominantly of round to oval, spindle-shaped, and poligonal cells. Specimens usually present a mixture of cell types and are characterized by dispersed pattern with loosely cohesive groups. Pseudofollicular arrangement may rarely be seen but follicles or papillary structures are not identified (62,63). The cytoplasm is slightly granular, and is usually configured as a
tard drop or cytoplasmic tail. Azurophilic cytoplasmic granules may be seen in air-dried smears. Nuclei are located eccentrically and are more large and pleomorphic than those of follicular cells (64,65). Bi- and multinucleation occur frequently, while the presence of nucleoli is not a consistent finding (62). A reliable confirmation of the cytologic diagnosis is obtained by immunocytochemistry with antibodies directed against CT. Positive reactivity of CT on cytologic smears was reported to correlate with MTC histology in 100% of cases (66,62). Immunocytochemical staining, however, may be difficult in samples with a poor cellularity. In these cases an US-guided core-needle thyroid biopsy may be useful (67).

5.2 Is thyroid cytology accurate for MTC?

Due to the dispersed pattern with pleomorphism, diagnostic surgery is frequently recommended even if a conclusive diagnosis is not made on FNA. (65). A few papers have analyzed the diagnostic accuracy of cytology in detecting MTC. FNA sensitivity for MTC is lower than for differentiated or anaplastic thyroid cancer (6,7). In a series of 44 cases, serum CT determination had higher sensitivity with respect to cytology (6). Only 20 nodules resulted cytologically suspicious for MTC (45% sensitivity), while 9 (20%) were reported as “undefined malignancy”. In a retrospective study on 77 MTC, FNA sensitivity was 63% while serum CT showed a sensitivity of 98%. Moreover, FNA was able to detect only 74.5% of MTC suspected on the basis of an elevated serum CT (7). It is of note that in both papers a non-negligible part of MTC was read as benign (up to 25% of MTC) or non conclusive (up to 9%) (6,7). Unpublished data of the Authors of this review display a 18% of histologically proved MTC having a benign and a 23% having a non conclusive cytologic report. With a different study design, Papaparaskeva et al (68) and Forrest et al (69) reviewed the aspirates reading of MTC and reported that this cancer was histologically confirmed in 81/91 (89% PPV) and 17/21 (81% PPV) cytologies, respectively. The need for surgery was, anyway, indicated in 99% of cases. A recent study confirmed a trend toward a better diagnostic accuracy for MTC of thyroid cytology. In a series of rare thyroid malignancies, it was reported a 83% sensitivity and a 100% predictive value for MTC using the Bethesda reporting system for thyroid cytology (70).

Based on the above papers (6,7,68-70), MTC may have a non conclusive report at FNA cytology. In clinical practice, the determination of serum CT in patients with nodules cytologically read as indeterminate should always be performed to exclude MTC.
6. CT measurement in FNA washout (FNA-CT)

6.1 What is the rationale for FNA-CT?

C cells produce CT and the measurement of CT in needle washout after biopsy is helpful in localizing MTC. The few papers that evaluated the role of FNA-CT for the diagnosis of MTC consistently showed that FNA-CT has a high accuracy (71-76).

6.2 When is the determination of FNA-CT indicated?

When a thyroid lesion is suspicious for MTC, FNA-CT should always be determined to prevent the risk of false negative cytologic reports. In multinodular goiters, FNA-CT may localize the MTC lesion and allows a better planning of the surgical approach. All patients with an elevated serum CT who undergo biopsy should perform a FNA-CT determination.

6.3 How to prepare and determine FNA-CT?

To date, there is no established method for FNA-CT sampling. Papers on FNA-CT used different assays and various modalities of preparation of the sample (e.g. washout in 1 ml of saline solution or analytical buffer) (71-76). This heterogeneous approach may lead to some discrepancy in the results. A recent paper (77) compared the use of saline solution and of specific CT-free buffer in patients without C cells disease. The results showed no significant difference between the two methods.

6.4 Is there an established cut-off level for FNA-CT?

Currently, there is no established cut-off of FNA-CT for the diagnosis of MTC. Arbitrary cut-off levels based on small series of patients were proposed by some authors at 36 pg/mL (71) or over 67 pg/ml (72). One paper reported that non-medullary nodules may have CT levels on FNA washout up to 8.5 pg/ml (77). It is noteworthy that MTC associated with a low FNA-CT value have never been reported.

7. RET (REarranged during Transfection) Mutations

7.1 Which is the clinical role of the genetic screening for RET?

Germline testing makes possible to differentiate hereditary from sporadic MTC cases. Up to 7% of patient with apparent sporadic MTC carry a germline RET mutation, and 2-9% of these are de novo ones (78-80). When the familial nature of MTC is assessed in the proband, RET genetic screening of the relatives reliably recognizes the asymptomatic
gene carriers of the identified RET mutation. In a recent report, a germline RET mutation was discovered in 6.5% of apparently sporadic MTC, and 58.2% of the relatives who resulted gene carriers had biochemical or clinical evidence of MTC (81). The identification of a specific RET mutation makes possible:
- to stratify patients into different categories for the risk of aggressive MTC
- to establish the indication and the timing for the screening of pheochromocytoma and hyperparathyroidism
- to select the timing for genetic screening of the relatives and for prophylactic thyroidectomy in those who result RET mutation carriers
- to reassure the relatives who do not carry RET mutations.

7.2 Which RET mutations should be investigated?

Over 98% of MEN 2A, 95% of MEN 2B and 85% of FMTC patients carry an identifiable germline RET mutation (82-84). RET mutations of MEN 2A and FMTC are more commonly located in exon 10 (codons 609, 611, 618, 620, 630) and exon 11 (codon 634). The latter is the most common, accounting for 80-85% of MEN 2A and 25-30% of FMTC (85). About 95% of MEN 2B patients carry mutation in codon 918 of exon 16, while the remaining 5% have codon 883 mutation or two-hit mutations of 804 codon with 805, 806 or 904.

In clinical practice a multiple-step approach should be followed (2). Testing of RET mutation should first include exons 10 and 11 and 13 through 16 (86). If genetic test results are negative, exons 5 and 8 should be examined as well (1).

7.3 When RET oncogene germinal mutation should be researched?

RET sequencing is needed in all cases of MTC. Even in the absence of family history of MTC, germline RET testing should be offered to:
- all patients with a personal history of primary CCH, MTC, or MEN2;
- all subjects with a family history of MEN2 or FMTC. In MEN2B the genetic screening should be done shortly after birth and in MEN2A and in FMTC before five years of age;
- patients with intestinal ganglioneuromatosis or lichen planus amyloidosis in the central upper area of their back as they are at risk to be a variant of MEN2A or FMTC with a 634 codon mutation (2).
Genetic analysis should be performed in all first-degree relatives of a MTC patient with an identified germline RET mutation. The potential gene carriers of the family should be tested before the recommended age of prophylactic thyroidectomy (2). Hirschsprung disease cosegregates with a few RET mutations for MEN2A/FMTC, even if at a low penetrance. In these cases the genetic screening should be directed to exon 10, codons 609, 611, 618, 620 (87).

7.4 Which role the RET genotype-phenotype correlation plays in MEN2 management?

A tight correlation is present between specific germline RET mutations, the age of onset of MTC and its aggressiveness (88,89). RET mutations may be stratified in 4 levels of risk for aggressive behaviour (2):
- Level D: highest risk for aggressive MTC (i.e. codons 918, 883, MEN2B). RET testing, thyroid ultrasound, serum CT determination, and prophylactic thyroidectomy are indicated as soon as possible, preferably within the first year after birth;
- Level C (codon 634, MEN2A): high risk for aggressive MTC. Children should undergo prophylactic thyroidectomy before the age of 5 years;
- Level B (codons 609, 611, 618, 620, 630): low risk for aggressive MTC;
- Level A (codons 768, 790, 791, 804, 891): least risk.

The recommended age for RET testing for levels C, B and A is before 3-5 years. The recommended age for initial thyroid US examination and the determination of serum CT is >3-5 years (Table 2). It has been suggested that in level A, B and C carriers of the RET gene, due to the heterogeneous phenotype and the risk of surgical complications in early childhood, serum levels of basal and stimulated CT should be used to personalize the timing of surgery. Thyroidectomy could be performed when stimulated CT becomes positive, independently of the type of RET mutation and the patient’s age. This approach was reported to offer a similar outcome when compared with the traditional timing of prophylactic thyroidectomy (90,91). Caution, however, is appropriate for level-C RET mutation gene carries and more data are needed to confirm this management approach.

7.5 What is the prognostic significance of the somatic mutations of RET oncogene in sporadic MTC?

Approximately 60 % percent of sporadic MTC have somatic mutations in their RET gene that are present in the tumor cells only and are undetectable by genetic testing on
leukocyte DNA. Somatic RET mutations occur at several codons (608, 618, 629, 630, 634, 649, 641, 918, 922) and most frequently at codon 918 (79,92-95). The presence of somatic RET mutations was correlated with a worse outcome of patients, as showed by a highest rate of cancer persistence and a lower survival rate (96). Somatic 918 mutation was associated with a more aggressive MTC behavior (97,98) and mutations in exons 15 and 16 of the RET gene are reported to correlate with lymph node metastases, persistent disease, and lower survival. In sporadic MTC patients who are positive for somatic RET mutation a close follow-up is appropriate. Somatic (acquired) RET mutations are still of uncertain prognostic significance and their routine research on tumor tissue is not suggested in clinical practice (1).

8. When coexisting tumors should be researched?

Patients with unknown mutational status or who have established germline RET mutations should be always tested for pheochromocytoma and hyperparathyroidism prior to thyroidectomy. In sporadic MTC patients who are negative for RET germline mutation, biochemical testing for coexisting tumors is not necessary (1).

Conclusions for a diagnostic approach in clinical practice

MTC is a potentially lethal tumor and its cure is strongly dependent by a well-timed diagnosis and appropriate treatment. Hence, a careful use and correct interpretation of the existing diagnostic tools are needed. Serum CT measurement represents the most sensitive test for an early diagnosis of MTC. Serum CT should always be tested in subjects with a family history positive for MTC, CCH, or MEN2. Thyroid US scan is a relevant diagnostic tool for differentiated thyroid carcinoma but is less predictive for MTC. Due to the imperfect diagnostic accuracy of US examination and the possible false-negative results of cytology by FNA, serum CT determination is suggested in patients with thyroid nodule features that warrant FNA biopsy, with an “indeterminate” cytologic report or who are undergoing thyroid surgery for any reason. For the same reasons, in patients undergoing nodule aspiration following the finding of an elevated serum CT, the CT determination in needle washout fluid should be performed. Elevated values of FNA-CT should be considered strongly indicative for MTC and prompt surgery. RET sequencing is mandatory in patients with MTC (even if apparently sporadic), CCH, or MEN2 and in all first-degree relatives of an MTC patient after the demonstration of a germline RET
mutation. In absence of a known mutation, a multiple-step approach should be followed for genetic testing.
### Table 1. Method-specific and gender-specific normal CT references for different assays.

<table>
<thead>
<tr>
<th>Assay</th>
<th>Manufacturer</th>
<th>Method</th>
<th>LoD</th>
<th>LoQ</th>
<th>Reference Range [pg/mL]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Elsa-hCT</td>
<td>Cis Bio</td>
<td>IRMA</td>
<td>1.5</td>
<td>5.5</td>
<td>m/f &lt;10, m &lt;9.54, f &lt;8.25</td>
</tr>
<tr>
<td>Immulite 2000</td>
<td>DPC</td>
<td>ICMA</td>
<td>2</td>
<td>5</td>
<td>m &lt;16, f &lt;8, m &lt;8.4, f &lt;5</td>
</tr>
<tr>
<td>Liaison II</td>
<td>DIASORIN</td>
<td>ICMA</td>
<td>1</td>
<td>3</td>
<td>m &lt;21.9, f &lt;11.1, m &lt;18.9, f &lt;5.5</td>
</tr>
<tr>
<td>Selco-CT</td>
<td>MEDIPAN</td>
<td>IRMA</td>
<td>1.6</td>
<td>-</td>
<td>m &lt;15, f &lt;10</td>
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### Table 2. RET genotype-phenotype correlation and MEN 2 management. Modified from Kloos (2).

<table>
<thead>
<tr>
<th>Codon</th>
<th>ATA risk level</th>
<th>MEN 2</th>
<th>MEN 2A</th>
<th>MEN 2°</th>
<th>MEN 2B</th>
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</thead>
<tbody>
<tr>
<td>515, 531, 600, 603, 777, 912</td>
<td>A</td>
<td>FMTC</td>
<td>Before 3-5 years</td>
<td>Before 3-5 years</td>
<td>Before 3-5 years</td>
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<tr>
<td>533, 649, 666, 768, 790, 791, 804, 891</td>
<td>A</td>
<td>FMTC/MEN 2A</td>
<td>Before 3-5 years</td>
<td>Before 3-5 years</td>
<td>Before 3-5 years</td>
</tr>
<tr>
<td>609, 611, 618, 620, 630, 631, 633</td>
<td>B</td>
<td>MEN 2A</td>
<td>Before 3-5 years</td>
<td>Before 3-5 years</td>
<td>Before 3-5 years</td>
</tr>
<tr>
<td>634</td>
<td>C</td>
<td>MEN 2°</td>
<td>Before 3-5 years</td>
<td>Before 3-5 years</td>
<td>Before 3-5 years</td>
</tr>
<tr>
<td>883, 918, 804+778, 804+805, 804+806, 804+904</td>
<td>D</td>
<td>MEN 2B</td>
<td>Before 3-5 years</td>
<td>Before 3-5 years</td>
<td>Before 3-5 years</td>
</tr>
</tbody>
</table>

- **Age of first serum CT**: Before 3-5 years<br>  >3-5 years<br>  >3-5 years<br>  >3-5 years<br>  >3-5 years
- **Age of first thyroid US**: Before 3-5 years<br>  >3-5 years<br>  >3-5 years<br>  >3-5 years<br>  >3-5 years
- **Age of prophylactic surgery**: Surgery may be delayed> 5 years if annual basal and stimulated CT, and annual US are normal
  - After 5 years if annual basal and stimulated CT, and annual US are normal
  - Before 1 year


34. Schlumberger M, Bastholt L, Dralle H, Jarzab B, Pacini F, Smit JWA. The European Thyroid Association Task Force 2012 European Thyroid Association Guidelines for Metastatic Medullary Thyroid Cancer. *Eur Thyroid J* 2012; 1:5–14


