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Is it time to give up calcitonin stimulation in C-cell disease or truly unravel its potential?

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Background: Calcitonin (Ctn) is a highly specific and sensitive marker for c-cell disease (CCD). In the era of precision medicine, preoperative differentiation between c-cell hyperplasia (CCH) and medullary thyroid cancer (MTC) facilitates a tailored surgical approach, avoiding over- or undertreatment.

Method: 451 patients with CCH or intrathyroidal MTC (TxN0M0), treated at two tertiary care centers between 01/2006 and 07/2025 were analyzed from a prospective database. Two correlating Ctn assays were employed for the biochemical diagnosis. All patients achieved postoperative biochemical cure. Gender- and assay-dependent basal and stimulated cut-off Ctn values as well as stimulated values after 2 and 5 minutes and carcinoembryonic antigen values (CEA) were analyzed and correlated with postoperative findings. The cohort was stratified by RET status.

Results: In the overall cohort, basal calcitonin (bCtn) showed the best diagnostic accuracy for preoperative discrimination of CCD and MTC. Stimulated calcitonin (sCtn) provided additional diagnostic benefit in male patients by achieving the highest positive predictive value (PPV) und specificity with a slightly lower area under curve (AUC) compared with bCtn (0.80 versus 0.81). In females with hereditary disease, sCtn showed the highest overall performance with an AUC, PPV and specificity of 0.83, 85% and 87%. CEA cut-offs and calcitonin peak timing did not add diagnostic value.

Conclusion: Combined preoperative assessment of basal and stimulated calcitonin testing may provide guidance in individual therapeutic decision-making to preoperatively stratify c-cell disease and tailor the therapeutic approach, helping to prevent over- or undertreatment. This appears to be particularly relevant in male patients in general and for females with hereditary CCD.

Ethical Approval

This study was conducted as a retrospective analysis of routinely collected clinical data. All data were fully anonymized prior to analysis. According to applicable institutional and national regulations, retrospective studies using anonymized data do not require formal review or approval by an institutional ethics committee. The study was conducted in accordance with the Declaration of Helsinki.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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Data Availability Statement

The data supporting the findings of this study are available from the corresponding author upon reasonable request.

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1 Introduction

Medullary thyroid carcinoma (MTC) is a rare neuroendocrine tumor, originating from the parafollicular c-cells of the thyroid gland[1]. In 2020, the incidence rate of thyroid cancer in Germany amounted to 8.6 per 100.000 individuals in women and 3.6 per 100.000 individuals in men, with 5 percent and 7 percent, respectively being MTCs, according to Robert-Koch-Insitute[2]. The curative standard therapy remains surgical, allowing a restrictive approach if MTC is detected in an early stage.

In the context of early diagnosis of c-cell disease (CCD), the concentration of calcitonin (Ctn) in the blood serum is considered a reliable parameter with a higher diagnostic sensitivity and specificity than the results of fine needle aspiration. A routine measurement of basal calcitonin as a part of the evaluation of thyroid nodules is therefore recommended, at least in European guidelines[1,3–8]. In case of elevated basal calcitonin levels, a determination of the stimulated calcitonin level can be carried out using calcium gluconate. This should serve the purpose of distinguishing between MTC and other (rare) non-thyroid-related causes of an elevated basal calcitonin level, to avoid an unnecessary extent of surgery[9–13].

Due to the introduction of highly specific novel immunochemiluminometric assays over the past decade, the added value of additional calcitonin stimulation tests for the definitive diagnosis of MTC with and without lymphogenic metastasis is being questioned and could not be proven in an analysis, conducted by Niederle et. al in 2020[14]. The role of Ctn stimulation in the biochemical grey zone with overlap between CCH and MTC remains less clearly defined. Calcitonin cut-offs do not adhere to a uniformly applicable standard, as they depend on the Ctn assay used. Published guidelines have identified concentrations ranging from 60 to 100 pg/ml as highly suggestive of MTC, regardless of the Ctn assay[9]. For the Siemens DPC assay, gender-specific cut-off groups for the biochemical detection of MTC were defined as follows: basal calcitonin (bCtn) concentrations > 23 pg/ml (specificity: 100%; sensitivity: 80%) or calcium-stimulated calcitonin (sCtn) concentrations > 780 pg/ml (specificity: 100%; sensitivity: 67%) for females and bCtn > 43 pg/ml (specificity: 100%; sensitivity: 58%) or sCtn > 1500 pg/ml (specificity: 100%; sensitivity: 54%) for males, respectively. A group with mildly calcitonin elevation (below the mentioned cut-offs) has also been delineated, in which MTC manifests with the maximum extent of pT1a. However, in this group there is a significant biochemical overlap with C-cell hyperplasia (CCH)[14,15]. The aim of this study is to compare preoperative bCtn values, sCtn values (after 2 and 5 minutes) and carcinoembryonic antigen values (CEA) with postoperative histologic results. The aim is to identify cut-offs for differentiating CCH from

MTC in patients with mildly elevated bCtn levels below established thresholds, supporting tailored surgical management and avoiding unnecessary surgery.

Patients and Methods

This study was designed to specifically correlate CCD with preoperatively obtained basal and stimulated Ctn levels through calcium infusion. Thus, rigorous inclusion and exclusion criteria were implemented to facilitate the understanding of the correlation between the Ctn dynamics and CCD:

1. Elevated gender-specific Ctn levels were present preoperatively.
2. A minimum of total thyroidectomy was conducted, with only primary operative cases included.
3. CCD (whether CCH or MTC) was diagnosed, based on pathological examination.
4. Biochemical cure was achieved, defined by undetectable postoperative bCtn levels
5. Patients with nodal or distant metastasis were excluded.
6. Only two correlating Ctn assays were used: the Siemens DPC assay and the Roche Cobas assay.
7. Patients with preoperative hypercalcemia and/or renal insufficiency were excluded to avoid false positive hypercalcitoninemia.

In total, 451 patients with CCH or MTC confined to the thyroid (TxN0M0) undergoing primary surgery between 01/2010 and 07/2025 in two high-volume university hospitals (Department of Visceral, Vascular and Endocrine Surgery, University Medicine Halle (UMH), Halle (Saale), Germany and the Department of General Surgery, Medical University of Vienna (MUV), Austria) were included and analyzed from two separate prospective databases.

Calcitonin and CEA analyses

At the Martin Luther University Halle-Wittenberg, the Immulite 2000® automated calcitonin assay (normal range <5.0 pg/ml for women and <8.4 pg/ml for men; Diagnostic Products Corporation, Los Angeles, CA) was used between June 2004 and July 2015. As of August 2015, the Elecsys® Calcitonin ECLIA assay (normal range <6.40 pg/ml for women and <9.52 pg/ml for men; Roche Diagnostics International, Rotkreuz, Switzerland) was used.

At the Medical University of Vienna, the Immulite 2000® automated calcitonin assay was used until January 2013, with the Elecsys® Calcitonin ECLIA assay being implemented afterwards.

Only these two assays were included, since the conversion is easily achieved due to the nearly ideal linear correlation using this equation (Immulite 2000® to Elecsys®: Ctn x 1.17 + 1.2)[16–18]. Biochemical cure at both institutions was defined when postoperative bCtn was below detection level (<2.0 pg/ml).

CEA serum levels were determined at both institutions using the monoclonal electrochemiluminescence immunoassay Elecsys (normal range <4.6 ng/ml; Roche Diagnostics, Mannheim, Germany).

Calcitonin stimulation through calcium injection (calcium stimulation test)

This test was done in accordance with the procedural proposal of Verburg et al. from 2013[16] . A blood sample for Ctn determination was drawn from a venous cannula of the fasting patient. This was labelled as basal Ctn (bCtn). Afterwards, a 10% calcium-gluconate solution (Calcium Braun 10%®; B. Braun Melsungen AG, Melsungen, Germany) at 2.5 mg/kg at a speed of 10ml/min was injected. Stimulated Ctn was determined at two and five minutes after calcium injection (2-min sCtn and 5-min sCtn); the peak Ctn level reached (whether after two or five minutes) was then labelled as sCtn.

Surgery

After obtaining written informed consent, all patients underwent primary total thyroidectomy. Central and lateral neck lymph node microdissection was performed selectively, based on preoperative Ctn levels, ultrasonographic findings and desmoplasia on frozen sections.

Pathological examination

All removed thyroid glands and lymph nodes underwent hematoxylin and eosin (HE) staining in addition to calcitonin immunohistochemistry. Histopathological diagnosis of CCH and MTC was performed in accordance with the criteria proposed by the World Health Organization[17]. For MTC, the staging system in accordance with the 8th edition of the American Joint Committee on Cancer (AJCC) TNM System was used.

Ethics Statement

Due to the retrospective design of the study, the requirement for informed consent was waived by the ethics committee. The study was conducted in accordance with the principles of the Declaration of Helsinki.

Statistical methods

Continuous data was presented as medians with interquartile ranges (IQR). A receiver operating characteristics (ROC) analysis stratified by patient's sex was conducted for bCtn, sCtn and CEA. The area under the curve (AUC) is a summary measure of the overall ability of the model to discriminate between the classes across all possible thresholds. A value of 1.0 indicates perfect discrimination between CCH and MTC, while a value of 0.5 implies no discriminative ability, equivalent to random chance.

Furthermore, separate sex-specific logistic regression models were formulated to differentiate CCH from MTC. Each model provided odds ratios (β), 95% confidence intervals (CI), and p-values. For each subgroup, five distinct models were developed using CEA, basal calcitonin levels, maximum stimulated calcitonin levels, and a combination of these as predictors. The odds ratio (OR) estimates indicate the increase in odds of MTC for each additional unit increase in the tumor marker. For each model, probabilities of MTC were estimated and used to generate ROC curves. These ROC curves for the univariate models align with prior ROC analyses conducted for the three tumor markers independently. For multivariate logistic regression models with two or three tumor markers, ROC curves were generated to determine an optimal cut-off for the prediction of MTC, which represents the combined predictive contribution of all included markers in the model. Consequently, the individual cutoffs of each marker cannot be isolated within the multivariate model. However, by examining the measured contributions of tumor markers in logistic regression, a threshold for weighted sum can be derived. This threshold defines a practical rule: if the weighted sum of tumor markers exceeds the cut-off, the patient is at high risk for MTC.

Results

451 patients (female: 200; male: 251) were included in this study. 262 (female: 91; male: 171) presented with postoperative findings of CCH and 189 with MTC (female: 109, male: 80). Among female patients with MTC, 82 were diagnosed with pT1a, 18 with pT1b and 9 with pT2 stage. Among male patients with MTC, 58 were diagnosed with pT1a, 16 with pT1b, 5 with pT2 and one with pT3 stage. For simplification, the single male patient with pT3 was grouped with the male pT2 cohort. No patient was diagnosed with MTC pT4 stage.

Apart from patients with a known hereditary background (RET proto-oncogene mutation), postoperative genetic testing was restricted to newly diagnosed MTC cases. In total 186 of 451 patients had a confirmed RET proto-oncogene status, of whom 57 (female: 31, male: 26) were positive, leaving 129 patients (female: 66, male: 63) with confirmed sporadic disease. Descriptive data for females and males are summarized in Table 1.

Biochemical analysis of the cohort

For female MTC patients, CEA levels increased progressively with stage advancement, showing median values of 3.0 ng/ml for pT1a, 9.4 ng/ml for pT1b and 44.9 ng/ml for pT2 in the overall cohort. This also applies for the sporadic MTC cohort with median CEA values rising from 5.2 ng/ml in pT1a to 14.7 ng/ml in pT1b to 39 ng/ml in pT2. For males, this pattern was not consistently observed, with median CEA levels of 2.65 ng/ml for pT1a, 11.0 ng/ml for pT1b and only 9.8 ng/ml for pT2-3 in the overall cohort, as well as with median CEA levels of 2.5 ng/ml for pT1a, 33.9 ng/ml for pT1b and 1.4 ng/ml for pT2 in the sporadic cohort.

For both females and males, the median bCtn and sCtn values were incremental with stage advancement in the overall cohort and in the sporadic cohort. In the overall cohort, for females, the median bCtn increased from 36 pg/ml to a median sCtn of 651pg/ml in pT1a, from 441 pg/ml to 6494 pg/ml in pT1b and from 1286 pg/ml to 23953 pg/ml in pT2 tumors. Whereas for males it was 32 pg/ml to 872 pg/ml in pT1a, 247 pg/ml to 3343 pg/ml in pT1b and from 1408 pg/ml to 55180 pg/ml in pT2-3 tumors.

In the sporadic cohort, for females the median bCtn increased from 36 pg/ml to a median sCtn of 692 pg/ml in pT1a, from 559 pg/ml to 3941 pg/ml in pT1b and from 1286 pg/ml to 5554 pg/ml in pT2 tumors. Whereas for sporadic males it was 32 pg/ml to 1070 pg/ml in pT1a, 177 pg/ml to 9683 pg/ml in pT1b and from 1026 pg/ml to 39410 pg/ml in pT2-3 tumors.

In the cohort with a RET germline mutation, the tumor stages of female and male patients with MTC did not exceed pT1a. Females showed a median CEA of 1.7 ng/ml and a median bCtn of 15 pg/ml, rising to a median sCtn of 323 pg/ml. Males presented with a median CEA of 3.2 ng/ml and a median bCtn of 23 pg/ml, rising to a median sCtn of 394 pg/ml. All medians of the RET cohort are considerably lower than in the sporadic cohort.

All corresponding data are presented in Table 2 and Table 3.

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Classification of CCH versus MTC ≥ pT1a based on sex-specific tumor marker cut-offs

To preoperatively predict CCH versus MTC, gender-specific ROC-analyses were conducted based on bCtn, sCtn and CEA. In addition, ROC-analyses were conducted exclusively for the cohorts with and without RET-germline mutations. The results are summarized in Table 3 and Table 4.

Overall cohort (RET-positive, sporadic, unknown RET status)

In females, a bCtn cut-off of 23 pg/ml was able to discriminate CCH from MTC best by achieving the highest AUC, PPV and specificity of 0.89, 94% and 94%, respectively. A sCtn cut-off of 407 pg/ml showed an underperformance with lower AUC, PPV and specificity of 0.86, 85% and 85%, respectively. The ROC-derived optimal CEA cut-off of 4.0 ng/ml showed the lowest AUC of 0.81 despite a high PPV and specificity of 92% and 94%.

In the male cohort, The calculated optimal bCtn cut-off was 27 pg/ml, showing a PPV of 65% and specificity of 82%. On the other hand, the ROC-derived calculated optimal sCtn cut-off of 812 pg/ml achieved the highest PPV and specificity of 78% and 92%, with a slightly lower AUC compared with bCtn (0.80 versus 0.81). CEA showed the lowest diagnostic accuracy with a calculated cut-off of 2.4 ng/ml, achieving an AUC, PPV and specificity of 0.74, 49% and 68%, respectively.

The calcitonin cut-offs for 100% PPV and 100% NPV:

Reaching 100% PPV implies that all positive test results are true positives (no false positives occur). However, this comes at the cost of reduced sensitivity, since stricter thresholds exclude borderline cases and thereby increase the number of false negatives. Conversely, achieving 100% NPV ensures that all negative test results are true negatives (no false negatives occur) but typically sacrifices specificity. The results of the calculation of 100% PPV and NPV are summarized in Table 5.

For the female overall cohort, 100% PPV for MTC was reached at bCtn of 121 pg/ml, sCtn of 1052 pg/ml or a CEA of 8.6 ng/ml. Conversely, MTC was ruled out (100% NPV) with a bCtn cut-off of 5 pg/ml, sCtn cut-off of 52 pg/ml or a CEA cut-off of 0.4 ng/ml.

For males, 100% PPV for MTC was reached at 111 pg/ml bCtn, 1976 pg/ml sCtn or 12.9 ng/ml CEA. MTC was ruled out (100% NPV) at cut-offs of 5 pg/ml for bCtn, 21 pg/ml for sCtn or 0.5 ng/ml for CEA.

Sporadic Cohort (n=129; female: 66, male: 63)

Female patients with sporadic CCD showed ROC-derived optimal bCtn and sCtn cut-offs at 29 pg/ml and 653 pg/ml, respectively. The CEA cut-off did not change (4.0 ng/ml). For male patients with sporadic disease, ROC-derived optimal cut-offs for bCtn, sCtn and CEA were 24 pg/ml, 874 pg/ml and 2.3 ng/ml, respectively. The bCtn cut-off for sporadic males was lower than for sporadic females (24 pg/ml versus 29 pg/ml).

In the female sporadic cohort, bCtn showed the highest overall performance with an AUC, PPV and specificity of 0.91, 100% and 100%. sCtn (AUC: 0.81, PPV: 93%, specificity: 90%) and CEA (AUC: 0.85, PPV: 100%, specificity: 100%) achieved comparable values to bCtn but did not match the overall performance of bCtn.

In the male sporadic cohort, bCtn achieved the highest overall performance (AUC: 0.81, PPV: 88%, specificity: 84%) but sCtn performed best, concerning PPV and specificity (AUC: 0.75, PPV: 96%, specificity: 96%). CEA showed the lowest diagnostic performance to discriminate CCD (AUC: 0.76, PPV: 84%, specificity: 81%).

RET Proto-Oncogene Cohort (n=57; female: 31, male: 26)

Female patients with hereditary disease showed ROC-derived optimal bCtn, sCtn and CEA cut-offs at 9 pg/ml, 182 pg/ml and 1.7 ng/ml, respectively. For male patients with hereditary disease, ROC-derived optimal cut-offs for bCtn, sCtn and CEA were 20 pg/ml, 123 pg/ml and 2.9 ng/ml, respectively. All cut-offs for the hereditary cohort were lower than for the overall and sporadic cohort.

In the female hereditary cohort, sCtn showed the highest overall performance with an AUC, PPV and specificity of 0.83, 85% and 87%. bCtn (AUC: 0.78, PPV: 76%, specificity: 73%) and CEA (AUC: 0.8, PPV: 89%, specificity: 93%) achieved comparable values but did not match the overall performance of sCtn.

In the male hereditary cohort, bCtn and sCtn achieved identical AUC values of 0.81. bCtn achieved 100% PPV and specificity, whereas sCtn showed lover PPV (64%) and specificity

(69%). While bCtn provided best PPV and specificity, sCtn achieved a higher sensitivity of 90% (versus 60%). CEA showed the lowest diagnostic performance to discriminate CCD (AUC: 0.67, PPV: 67%, specificity: 81%).

Analysis of the calcitonin stimulation dynamics: does it matter when peak stimulated calcitonin is reached ?

In the overall cohort, peak Ctn values were reached after two minutes after calcium injection in 55 patients (12%). A delayed peak calcitonin value attained after five minutes was observed in 12 of 91 (13%) female patients with CCH and 13 of 171 (8%) male patients with CCH, as well as in 18 of 109 (17%) female patients with MTC and 12 of 80 (15%) male patients with MTC.

This delayed calcitonin peak analysis offered no further diagnostic value in either sex. For females, PPV and NPV were 60% and 47%, and for males, 48% and 70%, respectively.

Discussion

The fascination behind calcitonin stimulation through different secretagogues has been an ongoing story since the early 1970's. This role has evolved from understanding the basic physiology of calcitonin through stimulation (and suppression), to enabling ''primitive'' commercial assays to detect calcitonin, until reaching the current era of precision medicine, where preoperative differentiation of c-cell disease (CCH vs. MTC) tailor its therapy, varying from a watch-and-wait strategy to individualized oncological resection.

While extrathyroidal calcitonin sources (e.g. prostate neoplasms, pheochromocytoma, pancreatic and pulmonary neuroendocrine neoplasms) are quite rare, they characteristically show a low to no stimulation response when injecting a calcitonin secretagogue, which is of great diagnostic value in the initial workup of elevated calcitonin levels[18].

For clinically advanced and/or metastatic thyroid cancer, imaging techniques in concordance with elevated calcitonin, with or without CEA are diagnostic for this MTC stage; hence limiting the diagnostic benefit of conducting a calcitonin stimulation test. In a recent literature review by Machens et al. the concept of 'desmoplasia' determination in frozen section for MTC

patients obviating the need for neck dissection in desmoplasia-negative MTC was emphasized[19]. This concept of desmoplasia determination is systematically implemented in both authors' institutions.

Thus, what remains to be conceptualized is tailoring the workup and the treatment of patients with mild calcitonin elevation but no signs of advanced or metastatic disease in the neck. Here, CCH with or without benign multinodular goiter, might as well mimic medullary thyroid cancer biochemically and morphologically, risking under- or more commonly overtreatment.

To address this specific question, the inclusion criteria aimed to select patients presenting with this diagnostic dilemma. Calcitonin (in its basal and calcium stimulated levels) and CEA were assessed separately and in non-hierarchical combination to predict CCD at hand.

In females, regardless of their RET-status, a bCtn cut-off of 23 pg/ml performed best in comparison to sCtn and CEA, indicating that the latter two markers added limited diagnostic benefit. Previous RET mutation exclusion offered a higher bCtn cut-off (29 pg/ml) with better diagnostic performance (AUC of 0.91 versus 0.89). In contrast, in females with hereditary disease, sCtn demonstrated the best overall diagnostic performance and therefore provided additional diagnostic value in this context.

The biochemical prediction of CCD in males in this series was overall poorer compared to females, except for bCtn in the hereditary cohort. For males, bCtn showed the best overall performance in predicting CCD but sCtn achieved the best PPV and specificity with an only marginally lower AUC compared with bCtn. Also, in the sporadic cohort sCtn showed consistently higher PPV and specificity than bCtn despite a poorer AUC. In the hereditary cohort, bCtn and sCtn showed comparable overall discrimination results, while sCtn offered value in reducing false-negative results.

In the overall cohort, enforcing a ROC-derived threshold yielding a PPV of 100% for MTC results in a reduced overall performance, reflected by a low AUC. In contrast, in selected subgroups, a 100% PPV was achieved at bCtn thresholds (29 pg/ml in sporadic females and 20 pg/ml in RET-positive males) that provided optimal AUC, indicating the diagnostic power of bCtn, especially in these two subgroups.

ROC-derived calculated optimal bCtn and sCtn cut-offs in this analysis are not in concordance with those proposed from same authors' institution by Niederle et al. back in 2020[20] with the exception of the female bCtn cut-off. Differences between the calculated ROC analysis cut-

offs in this study and those reported previously are likely attributable to a larger cohort size, as well as the exclusion of metastatic MTC in this cohort. In addition, the AUC of the proposed cut-offs attained in males for MTC and CCH by Niederle et al. were universally lower than their female counterparts, thus reiterating the findings in this study, showing that the biochemical prediction of CCD in males is generally less accurate.

In a German registry based study by Weber et al.[21] from 2022 with almost 40.000 patients included, the proposed bCtn cut-offs were much lower (7.9 pg/ml for females and 15 pg/ml for males) yielding a 100% NPV for females and 99.9% NPV for males. This essentially meant that missing a medullary thyroid cancer became almost impossible. However, the calcitonin assays used in that study were not listed and might not have been in linear correlation.

Conclusion

Combined preoperative assessment of basal and stimulated calcitonin testing may provide guidance in individual therapeutic decision-making to preoperatively stratify c-cell disease and tailor the therapeutic approach, helping to prevent over- or undertreatment. This appears to be particularly relevant for females with hereditary disease and for male patients in general. The question is therefore not whether calcitonin stimulation should be abandoned, but whether we are finally ready to fully harness its potential in individualized management of C-cell disease.

Table 1: Baseline patient cohort characteristics

	Female (n)	**Male (n)**
Overall cohort (n)	200	251
CCH	91	171
MTC	109	80
pT1a	82	58
pT1b	18	16
pT2	9	5
pT3	-	1
RET mutation (n)	31	26
CCH	15	16
MTC	16	10
MTC pT1a	16	10
MTC pT1b	-	-
MTC pT2	-	-
MTC pT3	-	-
Sporadic (n)	66	63
CCH	21	25
MTC	45	38
MTC pT1a	32	32
MTC pT1b	10	4
MTC pT2	3	2
MTC pT3	-	-
Calcitonin Assay (n)		
Siemens Immulite 2000	73	97
Roche Cobas Elecsys	183	98

Table 2: Biochemical characteristics of the female patient cohort

		CCH (overall)	MTC (overall)	MTC pT1a	MTC pT1b	MTC pT2-3
bCtn (pg/ml)	All	9.5 (0.5-120.6) IQR: 7-15	71.7 (4.8-4431-62) IQR: 19.8-203.9	35.5 (4.8-506.27) IQR: 14.9-87.5	440.5 (100.51-1491) IQR: 203.9-773	1286.0 (23.6-4431.62) IQR: 792.12-1444.61
	RET	7.8 (3.81-15.7) IQR: 4.55-13.7	15.3 (4.8-231) IQR: 9.51-24.85	15.3 (4.8-231) IQR: 9.51-24.85	-	-
	Sporadic	10 (6.0-28.1) IQR: 8-15)	74.0 (9.0-1824.1) IQR: 27.0-243.3	35.5 (9.0-243.3) IQR: 17.0-91.5	559.1 (194.3-1491) IQR: 301.1-867.0	1286.1 (792.1-1824.1) IQR: 792.12-1824.1)
sCtn (pg/ml)	All	177.8 (5.7-1051.5) IQR: 111.2-294.5	1416.3 (3.9-135601.5) IQR: 398.7 – 4449.4	651 (3.9-13247.9) IQR: 307.4-2119.7	6494.3 (1838.9-71413.3) IQR: 2324-27747	23952 (5554.0-135601.5) IQR: 12886.74-64251.96
	RET	111.2 (11.4-549.9) IQR: 44.8-151.2	322.9 (55.9-6651.0) IQR: 141.9-518.5	322.9 (55.9-6651) IQR: 141.88-518.5	-	-
	Sporadic	453.0 (121.0-836.6) IQR: 222.1-517.1)	1374 (3.88-71413.32) IQR: 525-4169.91	692 (3.88-13247.94) IQR: 444-2239	3941 (1986-71413.3) IQR: 2324-27747	5554.0 (5554.0-30629.5) IQR: 5554.0-30629.5)
CEA (ng/ml)	All	1.4 (0.2-8.5) IQR: 1.0-2.0	5.45 (0.4-155.5) IQR: 1.8-10.1	3 (0.4-22.6) IQR: 1.6-6.5	9.4 (0.8-116.8) IQR: 6.5-15.5	44.85 (23.6-155.5) IQR: 32.1-68.25
	RET	1.05 (0.2-2) IQR: 0.5-1.4	1.7 (0.9-10.1) IQR: 1.2-3.7	1.7 (0.9-10-1) IQR: 1.2-3.7	-	-
	Sporadic	1.55 (0.8-3.8) IQR: 1.1-2.5	6.2 (0.4-116.8) IQR: 2.4-13.8)	5.15 (0.4-22.6) IQR: 1.95-6.75	14.65 (4.1-116.8) IQR: 9.3-24.7	38.5 (35.3-53.2) IQR: 35.3-53.2)

*Values are presented as Median (minimum-maximum); IQR = interquartile range

Table 3: Biochemical characteristics of the male patient cohort

		CCH (overall)	MTC (overall)	MTC pT1a	MTC pT1b	MTC pT2-3
bCtn (pg/ml)	All	17.1 (0.7-110.9) IQR: 12.0-23.8	51.0 (4.7-3918.4) IQR: 24.0-155.2	31.8 (4.7-370.5) IQR: 364.0-1632.2	247.3 (95.0-3918.4) IQR: 123.1-560.3	1408.3 (54.0-1998.0) IQR: 853.8-1869.3
	RET	9.3 (0.74-17.4) IQR: 5.7-12.8	22.8 (6.7-95.1) IQR: 7.9-28.0	22.8 (6.7-95.1) IQR: 7.86-28)	-	-
	Sporadic	18.8 (9.0-51.51) IQR: 15-22.26	40.8 (11.3-3918.4) IQR: 23.8-80.3	32.1 (11.3-370.5) IQR: 21.1-60.3	177.0 (102.0-3918.4) IQR: 109.3-2078.0	1026.0 (54.0-1998.1) IQR: 54.0-1998.1
sCtn (pg/ml)	All	177.8 (5.7-1051.5) IQR: 111.2-294.5	1196.8 (21-76353.9) IQR: 409.9-3517.0	871.5 (21.0-15214.3) IQR: 364.1-1632.2)	3343.3 (140.1-61888.4) IQR: 1550.5-5591.8	55180.4 (3511.0-76353.9) IQR: 16439.3-75308.1
	RET	108.5 (5.4-1215) IQR: 62.8-253.5	394.0 (94.4-1698.1) IQR: 150.96-476.22	394.0 (94.4-1698) IQR: 151.0-476.2	-	-
	Sporadic	477.0 (158.0-1336.2) IQR: 374.4-703.0)	1153.0 (161.1-75308.1) IQR: 440-2046.36	1069.5 (161.1-5556.0) IQR: 432.5 – 1638.6	9682.7 (386.3-61888.4) IQR: 2637.7-38182.4	39409.5 (3511-75308) IQR: 3511-75308
CEA (ng/ml)	All	1.8 (0.3-12.8) IQR: 1.1-2.7	3.1 (0.5-57.5) IQR: 1.8-6.4	2.65 (0.5-16.4) IQR: 1.8-4.25	11.0 (2-57.7) IQR: 4-14.6	9.8 (1.4-45.8) IQR: 1.9-31.5
	RET	1.6 (0.3-6.9) IQR: 0.9-2.5	3.15 (0.5-6.4) IQR: 1.3-4	3.15 (0.5-6.4) IQR: 1.3-4	-	-
	Sporadic	1.7 (0.3-6.7) IQR: 1.1-2.1	2.8 (0.9-57.7) IQR: 1.8-6.1	2.5 (0.9-16.4) IQR: 1.8-4.3	33.9 (5.7-57-7) IQR: 8.4-57.3	1.4 (1.1-1.4) IQR: 1.4-1.4

***Values are presented as Median (minimum-maximum); IQR = interquartile range**

Table 3: Tumor marker cut-off analysis (CCH versus MTC) of the female patient cohort

	Cut-off	AUC	PPV (%)	NPV (%)	Specificity (%)	Sensitivity (%)
All						
bCtn (pg/ml)	≥23	0.89	94	74	94	72
sCtn (pg/ml)	≥407	0.86	85	75	85	75
CEA (ng/ml)	≥4.0	0.81	92	64	94	57
Sporadic cohort						
bCtn (pg/ml)	≥29	0.91	100	64	100	73
sCtn (pg/ml)	≥653	0.81	93	58	90	66
CEA (ng/ml)	≥4.0	0.85	100	52	100	68
RET cohort						
bCtn (pg/ml)	≥9	0.78	76	79	73	81
sCtn (pg/ml)	≥182	0.83	85	72	87	69
CEA (ng/ml)	≥1.7	0.80	89	65	93	53

Table 4: Tumor marker cut-off analysis (CCH versus MTC) of the male patient cohort

	Cut-off	AUC	PPV (%)	NPV (%)	Specificity (%)	Sensitivity (%)
All						
bCtn (pg/ml)	≥27	0.81	65	86	82	73
sCtn (pg/ml)	≥812	0.80	78	84	92	64
CEA (ng/ml)	≥2.4	0.74	49	81	68	67
Sporadic cohort						
bCtn (pg/ml)	≥24	0.81	88	70	84	76
sCtn (pg/ml)	≥874	0.75	96	63	96	63
CEA (ng/ml)	≥2.3	0.76	84	61	81	66
RET cohort						
bCtn (pg/ml)	≥20	0.81	100	80	100	60
sCtn (pg/ml)	≥123	0.81	64	92	69	90
CEA (ng/ml)	≥2.9	0.67	67	76	81	60

449 **Table 5: ROC-derived threshold yielding a PPV or NPV of 100% in the overall cohort**
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	Cut-off	AUC	PPV (%)	NPV (%)	Specificity (%)	Sensitivity (%)
Female cohort (PPV 100%)						
bCtn (pg/ml)	≥121	0.64	100	55	100	33
sCtn (pg/ml)	≥1052	0.76	100	66	100	55
CEA (ng/ml)	≥8.6	0.63	100	55	100	33
Female cohort (NPV 100%)						
bCtn (pg/ml)	≤5	0.59	57	100	9	100
sCtn (pg/ml)	≤52	0.61	58	100	17	100
CEA (ng/ml)	≤0.4	0.58	57	100	6	100
Male cohort (PPV 100%)						
bCtn (pg/ml)	≥111	0.78	100	76	100	33
sCtn (pg/ml)	≥1976	0.80	100	77	100	36
CEA (ng/ml)	≥12.9	0.72	100	71	100	11
Male cohort (NPV 100%)						
bCtn (pg/ml)	≤5	0.33	32	100	2	100
sCtn (pg/ml)	≤21	0.33	32	100	2	100
CEA (ng/ml)	≤0.5	0.33	32	100	3	100

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