Received: 8 August 2023 | Revised: 24 October 2023 | Accepted: 25 October 2023

DOI: 10.1002/cncy.22779

ORIGINAL ARTICLE

An old friend, a new insight: Calcitonin measurement in serum and aspiration needle washout fluids significantly increases the early and accurate detection of medullary thyroid cancer

Berna Evranos Ogmen MD[1] | Nurcan Ince MD[1] | Aysegul Aksoy Altınboga MD[2] | Leyla Akdogan MD[1] | Sefika Burcak Polat MD[1] | Birgul Genc[3] | Ebru Menekse MD[4] | Cevdet Aydin MD[1] | Oya Topaloglu MD[1] | Reyhan Ersoy MD[1] | Bekir Cakir MD[1]

[1]Department of Endocrinology and Metabolism, Ankara Yildirim Beyazit University, Faculty of Medicine, Ankara Bilkent City Hospital, Ankara, Turkey

[2]Department of Pathology, Ankara Yildirim Beyazit University, Faculty of Medicine, Ankara Bilkent City Hospital, Ankara, Turkey

[3]Department of Endocrinology and Metabolism, Ankara Yildirim Beyazit University, Health Sciences Institute, Ankara Bilkent City Hospital, Ankara, Turkey

[4]Department of Surgery, University of Health Sciences, Faculty of Medicine, Ankara Bilkent City Hospital, Ankara, Turkey

Correspondence
Berna Evranos Ogmen.
Email: evranosberna@gmail.com

Abstract

Background: The sensitivity of cytological (CY) evaluation after fine-needle aspiration (FNA) for detecting medullary thyroid carcinoma (MTC) is a subject of controversy. The routine use of serum calcitonin (CT) in patients with thyroid nodules is not universally adopted. The authors conducted CT screening of FNA washout fluid (FNA-CT) to address the diagnostic challenges. The objective was to assess the contributions of serum CT, FNA cytology (FNA-CY), and FNA-CT to the diagnosis.

Methods: Between February 2019 and June 2022 (group 1), the authors prospectively screened the CT of patients with thyroid nodules. Both FNA-CY and FNA-CT were performed for patients with persistently elevated CT values. The sensitivity of FNA-CY, serum CT, and FNA-CT for accurate diagnosis was evaluated. Additionally, the authors retrospectively examined data from patients with thyroid nodules before CT screening (2008–2019) (group 2). They compared the characteristics of MTC patients in groups 1 and 2.

Results: MTC was identified in 30 patients (0.25%) in group 1 and 19 (0.07%) in group 2. A FNA-CT cutoff value of 4085.5 pg/mL detected MTC with a sensitivity of 96.8%, and a serum CT cutoff value of 28.3 pg/mL detected MTC with a sensitivity of 86.7%. In contrast, FNA-CY detected MTC with a sensitivity of 42.4%. In group 1, 18 patients (60%) with MTC were diagnosed with microcarcinoma, whereas only two patients (10.5%) in group 2 had microcarcinoma.

Conclusions: This study detected MTC earlier by routinely measuring serum CT in all patients with nodular thyroid disease and performing FNA-CT in those with elevated values. FNA-CT and serum CT sensitivities were significantly higher than

those of FNA-CY. This study revealed different FNA-CT cutoff values compared to other studies, emphasizing the need for determining clinic-specific cutoff values.

KEYWORDS
calcitonin, calcitonin in washout fluid of fine-needle aspiration, fine-needle aspiration cytology, medullary thyroid cancer, sensitivity

INTRODUCTION

Medullary thyroid carcinoma (MTC) is a neuroendocrine malignancy originating from parafollicular C cells. Sporadic or isolated MTC accounts for 75% of cases and the remaining 25% are hereditary. Hereditary cases are familial or part of multiple endocrine neoplasia type 2, an autosomal-dominant syndrome caused by germline-activating mutations in the *RET* proto-oncogene.[1] Calcitonin (CT) production is a characteristic feature of this tumor. However, several technical factors can affect the CT assay, and other nonthyroidal pathologic conditions can increase CT,[2,3] thus limiting its routine use in clinical practice. The American Thyroid Association (ATA) task force has confirmed that they could not make recommendations for or against the routine measurement of CT.[4] Furthermore, the American Association of Clinical Endocrinologists/Associazione Medici Endocrinologi/European Thyroid Association (AACE/AME/ETA) guidelines have suggested examining CT only in subjects with a familial history of MTC and patients with cytology suggestive of MTC or undergoing surgery for goiter.[5]

Cytological evaluation after fine-needle aspiration (FNA) is the most reliable tool for assessing thyroid nodules, but its sensitivity in detecting MTC is controversial.[6–8] The Revised ATA Guidelines for the Management of Medullary Thyroid Carcinoma advise that thyroid nodules measuring 1 cm or larger should undergo evaluation through FNA cytology (FNA-CY) depending on the ultrasound (US) characteristics.[9] Moreover, if FNA-CY is either inconclusive or suggestive of MTC, CT should be measured in the FNA washout fluid (FNA-CT) (Grade B recommendation).[9]

Specific US characteristics can give rise to suspicion of MTC. MTC nodules are typically hypoechoic, often hypervascular.[10] Punctate echogenic foci are relatively common and may correspond to smaller amyloid deposits rather than true microcalcifications.[11] MTC nodules exhibit nonparallel orientation and irregular borders more frequently than benign lesions, although these features are not highly relevant in diagnosing MTC. Suspicious sonographic papillary thyroid cancer (PTC) features can also be observed in MTC but with lower frequency.[10,12,13] Moreover, some classifications suggest that up to one-third of MTCs may exhibit a "non-malignant" sonographic pattern.[14,15]

The most recent AACE/ACE/AME guidelines indicate that FNA-CT could be used for suspicious thyroid nodules in patients at risk for MTC or multiple endocrine neoplasia type 2 syndrome. At the same time, FNA-CT must be used in cervical lymph nodules suspicious of metastatic MTC.[5] ATA[9] and AACE/AME/ETA[16] guidelines suggest using FNA-CT in cervical lymph nodules suspicious of metastatic MTC. Regarding using FNA-CT in thyroid nodules, the main unresolved issue might be the selection of patients who need to undergo FNA-CT. A non-negligible rate of MTC delays the diagnosis due to the limitation of cytology and the lack of universal consensus to evaluate serum CT in thyroid nodule patients.

The AACE/ACE/AME guidelines define cutoff points of 26 and 68 pg/mL for basal serum CT to separate non-MTC patients from MTC for females and males, respectively.[5] In contrast, other guidelines have not specified cutoff points.[4,9] The ATA guidelines do not report a specific cutoff level for FNA-CT that has to be adopted.[4,9] AACE/AME/ETA guidelines have established that FNA-CT levels of lymph nodes >50 pg/mL should be regarded as suspicious, and values >100 pg/mL are nearly diagnostic of metastatic MTC.[16] These guidelines have not reported specific items for using FNA-CT in thyroid nodules.

The present study examined patients with nodular thyroid disease whose serum CT was routinely measured, and FNA-CT was performed in elevated cases. This research aimed to assess serum CT, FNA-CY, and FNA-CT and their contribution to diagnosis. The clinical data of MTC patients before and after these methods were applied have also been compared to evaluate the importance of measuring serum CT and FNA-CT.

MATERIALS AND METHODS

This study compared the detection of MTC using two different models in one cohort from 2019 to 2022 (group 1) and another from 2008 to 2019 (group 2). The institutional ethics committee of Ankara Bilkent City Hospital has approved the study. Thyroid US was performed on all patients with a high-frequency (10–15 MHz) linear array transducer (Hitachi Hivision Avius with EUP-L74 M, Hitachi Aloka Medical, Tokyo, Japan). In group 1 (new method), data were collected prospectively. CT was screened from 12,030 patients with well-documented thyroid nodules (any size, even <1 cm in diameter) who were treated at the endocrinology polyclinics of Ankara Bilkent City Hospital between February 2019 and June 2022. For patients who presented for follow-up, had persistently elevated (exceeding reference values for over 3 months) serum CT levels, and consented for biopsy, FNA biopsies were collected for FNA-CY and FNA-CT. Patients with elevated serum CT elevation due to confounding factors such as smoking, alcohol consumption, proton pump inhibitor use, bacterial infection, hypercalcemia, and renal insufficiency were

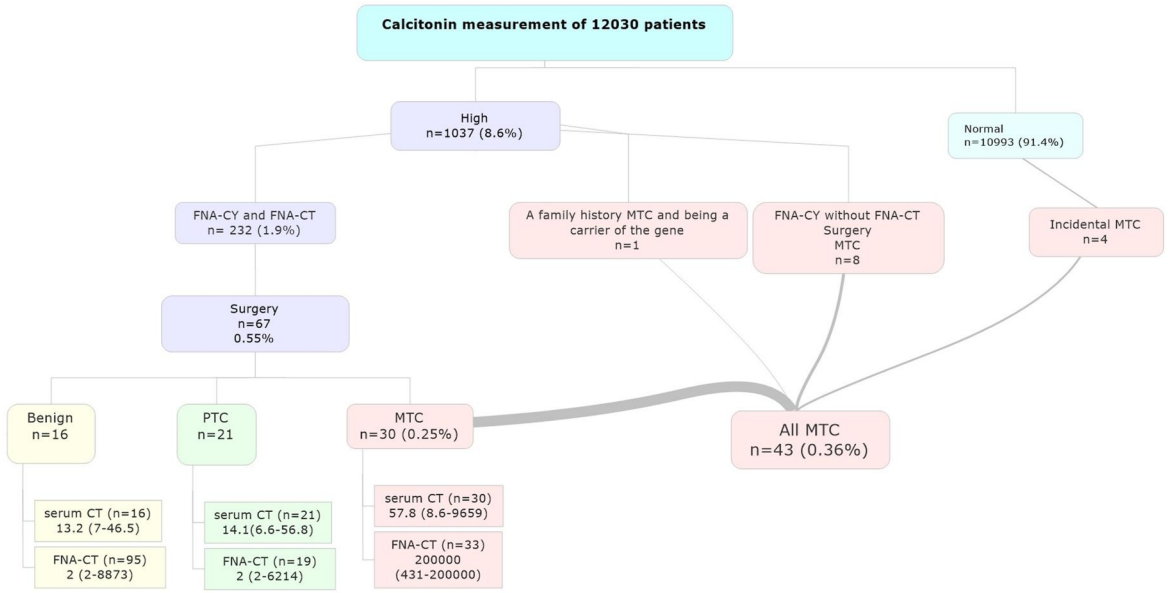

FIGURE 1 The data include patients from group 1 and any additional cases of medullary thyroid cancer detected between February 2019 and June 2022.

excluded. Patients with a familial history of MTC or prior malignant or suspicious for malignancy cytology were excluded (Figure 1).

All nodules measuring ≥1 cm, and nodules smaller than 1 cm displaying at least one of the suspicious US characteristics,[17] including a taller-than-wide shape, irregular margins, microcalcifications, and marked hypoechogenicity, were subjected to FNA. For patients lacking nodules larger than 1 cm, nodules exhibiting at least one of these suspicious US features underwent FNA. In cases where none of these features were present, the largest and most accessible nodules were evaluated. FNA was performed under US guidance (Logic Pro 200 GE and 7.5 MHz probes; Kyunggigo, Korea) with 22–25 gauge needles. All patients gave informed consent before the FNA procedure.

After smear preparation of specimens for cytological analysis, the same needle and syringe were quickly washed in 1 cc of saline solution and used for CT measurement. Experienced cytologists in thyroidology examined smears and were unaware of the results of serum CT and FNA-CT. Cytological findings were classified according to the Bethesda System as nondiagnostic (ND), benign, atypia of undetermined significance (AUS), follicular neoplasm (FN), suspicious for malignancy (SFM), and malignant, which were categorized as 1 to 6, respectively.[18,19]

Data from 67 patients who underwent thyroidectomy based on the FNA-CY and/or FNA-CT results had follow-up at our institution and were included in this study. Thyroid pathologies were categorized into benign, PTC, and MTC. Nodules that underwent FNA were compared with the pathology results and categorized into one of these three pathologies. Pathology results that did not correspond to a nodule that had undergone FNA were assessed as incidental findings. Furthermore, additional CT immunostains were conducted to exclude MTC when nodules exhibited high FNA-CT levels with nonmedullary pathology. MTC was further classified into microcarcinoma (micro-MTC, ≤10 mm) or macrocarcinoma (macro-MTC, >10 mm) based on the largest dimension observed in the pathology results.

Moreover, we analyzed the pathology outcomes from 2126 patients with thyroid conditions who underwent thyroidectomy for various reasons at our hospital between February 2019 and June 2022. For this study, patients diagnosed with MTC were included. Consequently, we retrospectively assessed patients with MTC who had not undergone FNA-CT to enhance the likelihood of identifying the prevalence of MTC.

In group 2 (old method), data were assessed retrospectively. This group included 25,205 patients with nodular thyroid disease who received treatment at our endocrinology clinic from January 2008 to February 2019. All nodules measuring ≥1 cm, as well as nodules <1 cm with at least one of the suspicious US features, underwent FNA. A diagnosis was obtained through presurgical FNA-CY and post-surgical histological examinations to diagnose MTC in these patients. Serum CT measurement was exclusively conducted for patients falling under the Bethesda category 5 or 6 based on cytology results.

The blood sample was collected from all patients to measure free thyroid hormones, TSH, thyroid antibodies, and CT. For the CT analysis, our laboratory used a Siemens IMMULITE 2000 automated chemiluminescence immunoassay analyzer with a sensitivity of 2 pg/mL and reference values of up to 5 pg/mL for women and 8.4 pg/mL for men. The analysis was performed using a standard assay kit designed for in vitro diagnostics provided by Siemens Healthcare Diagnostics Products Limited. We conducted a comparison of the characteristics of MTC patients in both group 1 and group 2.

Statistical analysis

Statistical analyses were performed using the IBM Statistical Package for Social Sciences for Windows ver. 25.0 (IBM Corp., Armonk, New York). The Kolmogorov–Smirnov test has evaluated the normality of data distribution. Moreover, the χ^2 and Fisher exact tests for the categorical variables have been used. The Student *t*-test for parametric variables and the Mann–Whitney *U* test for nonparametric variables have been performed to compare outcomes between two independent groups. In addition, the Kruskal–Wallis test has been employed to assess the significance of differences among the means of three or more independent groups. All *p* values have been two-sided, and a $p < .05$ level has been considered statistically significant. The true positive (TP) and true negative (TN) have been defined as the correct prediction of the presentation of MTC. Sensitivity = TP/(TP + FN). TP was considered when FNA-CY was reported as SFM or malignant. Finally, receiver operating characteristic curve analysis has been performed to measure the diagnostic accuracy and the optimal cut-point value of serum and FNA CT.

RESULTS

The serum CT of 12,030 patients was measured in group 1, and CT was high in 1037 (8.6%) patients (Figure 1). FNA-CY and FNA-CT were performed on 456 nodules of 232 patients (Figure 2). Of the FNA-CY results, 87 (19.1%), 287 (63%), 54 (11.8%), 16 (3.5%), and 12 (2.6%) were ND, benign, AUS, SFM, and malignant, respectively (Figure 2).

There were 67 patients with 147 nodules operated on, consisting of 38 females and 29 males. Their median age was 56 years, ranging from 21 to 76 years. A total of 30 patients had MTC, 21 had PTC, and 16 had benign pathology. PTCs smaller than 5 mm were incidentally detected in four patients. MTC was detected in 33 nodules of 30 patients. There were five ND, seven benign, seven AUS, eight SFM, and six malignant nodules diagnosed as MTC.

Serum CT values were compared according to pathology results. The medians were 57.8 pg/mL (range, 8.6–9659) in the MTC group, 14.1 pg/mL (range, 6.6–56.8) in the PTC group, and 13.2 pg/mL (range, 7–46.5) in the benign group ($p < .001$) (Figure 1). Moreover, serum CT was lower than 100 pg/mL in 17 (56.6%) patients with MTC. Median FNA-CT was 200,000 pg/mL (range, 431–200,000) in nodules with MTC, 2 pg/mL (range, 2–6214) in nodules with PTC, and 2 pg/mL (range, 2–8873) in benign nodules ($p < .001$). FNA-CY and pathology results of the nodules are shown in Figure 2. Although the cytology of the nodules with MTC was different between Bethesda categories, the median FNA-CT was high and similar in all categories ($p = .084$) (Figure 3). There were 18 (60%) patients with MTC who had microcarcinoma detected in pathology sections. The median size of MTC was 0.9 cm (range, 0.1–5.5).

The sensitivity of the FNA-CY was calculated as 42.4% (14 of 33). The cutoff value of serum CT for MTC was calculated as 28.3 pg/mL with a sensitivity of 86.7% and a specificity of 89.2%. The area under the curve was 0.923 (95% confidence interval [CI], 0.853–0.994; $p < .001$). The cutoff value of FNA-CT for MTC was calculated as 4085.5 pg/mL with a sensitivity of 96.8% and a specificity of

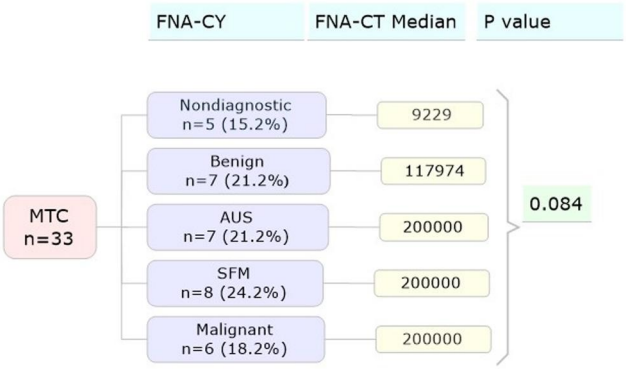

FIGURE 3 Comparison of fine-needle aspiration calcitonin with fine-needle aspiration cytology findings in patients diagnosed with medullary thyroid cancer.

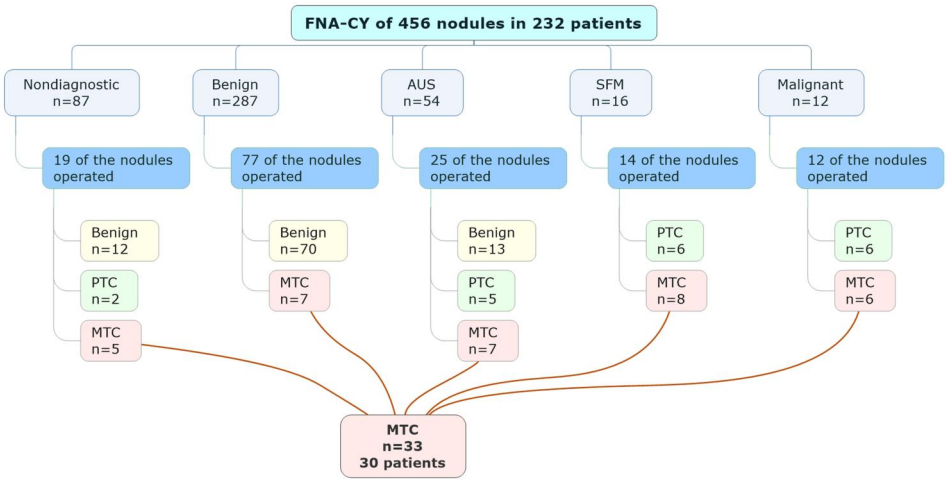

FIGURE 2 Fine-needle aspiration cytology and pathology findings of patients.

97.3%. The area under the curve was 0.995 (95% CI, 0.987–1; $p < .001$.

The MTCs in all thyroidectomized patients in our hospital in 2019–2022 were retrospectively evaluated. FNA-CT was not performed for eight patients with MTC due to very high CT values with Bethesda category 5 or 6. FNA-CT was not performed on a patient with elevated serum CT who was a gene carrier with a history of familial MTC. MTC was detected incidentally in four patients with normal serum CT levels. The largest diameter of incidentally detected MTCs was 5 mm. Finally, MTC was detected in 43 (0.36%) of 12,030 patients (Figure 1).

In group 2, 19 (0.07%) of 25,205 patients had confirmed MTC after surgery. Two (10.5%) of the patients had micro-MTC. The median serum CT was 994 pg/mL (range, 51.2–16500). Age and gender were similar among patients with MTC in groups 1 and 2. The tumor size and CT level of patients with MTC were more extensive in group 2 than in group 1 ($p < .001$) (Table 1).

DISCUSSION

In this study, serum CT levels were measured in all patients with nodular thyroid disease who applied to our endocrinology clinic between February 2019 and June 2022. For those with elevated results, we assessed the contribution of FNA-CY, serum CT, and FNA-CT in identifying MTC. A 4085.5 pg/mL cutoff value for FNA-CT detected MTC with a sensitivity of 96.8%, whereas a cutoff value of 28.3 pg/mL for serum CT identified MTC with a sensitivity of 86.7%. In contrast, FNA-CY detected MTC with a sensitivity of 42.4%. We also questioned whether this new approach altered the results compared to the old method. The new method allowed researchers to detect more patients, even when they had micro-MTCs with lower serum CT levels.

The findings of this study validate previous reports[20] and, in a more extensive patient cohort, affirm that screening for nodular thyroid disease using CT measurement is a sensitive method for diagnosing unsuspected MTC. A recent meta-analysis encompassing 17 trials and 74,407 patients with nodular thyroid disease found the prevalence of MTC to range from 0.11% to 0.85%, corroborating the results of this study.[21] This prevalence variation was influenced by factors such as patient selection, the number of patients, CT measurement methodology, technical aspects related to different CT measurement kits, and differences in the definition of the normal range for serum CT. Notably, the historical MTC prevalence in our endocrinology clinic was lower than the rates indicated in the meta-analysis.[21] The authors of this study propose that the decision not to measure serum CT in every patient with nodular thyroid disease reduces the likelihood of an MTC diagnosis rather than representing a change in the frequency of MTC over time. Incorporating serum CT measurement and FNA-CT has increased MTC detection in our clinic by at least 5-fold.

In this study, FNA-CY detected only 42.4% of the MTC cases accurately. US-guided FNA-CY is highly recommended for distinguishing between benign thyroid nodules and malignant thyroid neoplasms. However, its diagnostic performance for MTC may not be as robust. A meta-analysis of 15 relevant studies revealed that FNA-CY could identify only approximately half of the 641 MTC lesions.[6] Furthermore, a recently published meta-analysis encompassing six studies demonstrated that FNA-CY sensitivity varied from 20% to 86%, with an average value of 54% and notable heterogeneity.[8] The relatively high rate of false-negatives can be attributed to the morphological diversity of MTC. Additionally, micro-MTC exhibits cytological characteristics such as fewer oncocytic changes and more colloid presentation.[7,22–24] It is worth noting that the sensitivity of FNA-CY in the most recent studies[25,26] has improved compared to earlier publications. Consequently, cytopathologists have become more attuned to this challenging diagnosis, possibly influenced positively by the concurrent use of FNA-CT. Nevertheless, it is expected that with increased awareness, the sensitivity of FNA-CY in MTC diagnosis will improve over time. This part of the study was conducted prospectively, with cytologists unaware of the results of serum CT and FNA-CT. This could explain the relatively low sensitivity observed in this study, which aligns with previous research.

The results of this study showed that using an FNA-CT cutoff value of 4085.5 pg/mL had a high sensitivity of 96.8% and specificity of 97.3% when detecting MTC. Boi et al.[27] assessed the diagnostic performance of FNA-CT with a cutoff of 36 pg/mL, which corresponded to three times the highest FNA-CT concentration observed in benign cervical lesions. Kudo et al.,[28] Diazzi et al.,[29] Trimboli et al.,[30] and De Crea et al.[31] identified MTC with cutoff values of 67 pg/mL, 17 pg/mL, 39.6 pg/mL, and 10.4 pg/mL, respectively.

The study by Boi et al.[27] did not specify the number of control patients from whom the cutoff data were determined. Trimboli et al.[30] derived their cutoff value from control measurements from

TABLE 1 Comparison of the patients with MTC in groups 1 and 2.

	MTC patients, group 1, $n = 30$	MTC patients, group 2, $n = 19$	p
Age (years)	55.6 ± 13.1	49.9 ± 14.4	.14
Female/male	21/9	12/7	.61
Largest tumor size (cm), median (range)	0.9 (0.1–5.5)	2.4 (1–6.5)	<.001
Micro MTC, No. (%)	18 (60)	2 (10.5)	.001
Serum CT (pg/mL), median (range)	57.8 (8.6–9659)	994 (51.2–16500)	<.001

Abbreviations: CT, calcitonin; max, maximum; Micro MTC, medullary thyroid microcarcinoma; min, minimum; MTC, medullary thyroid cancer.

four centers. However, these studies emphasized determining a unique cutoff value tailored to individual centers. The authors of these studies have also reported detectable levels of FNA-CT (higher than the functional sensitivity of the method used) in nonmedullary thyroid nodules. Recently, Trimboli et al.[8] conducted a meta-analysis of six studies, including some of the abovementioned studies. They highlighted the primary limitation of FNAB-CT as the absence of a standardized threshold for clinical application, suggesting that using an institutional cutoff could address this issue.

CT is produced by parafollicular C cells in the thyroid gland, and CT levels in the normal thyroid and thyroid nodules other than MTC may show some elevation. It is possible that C cells could be entrapped in the FNA sample, particularly in the middle-upper thyroid lobes.[32–34] Moreover, C-cell hyperplasia can be present in conditions like Hashimoto's thyroiditis and colloid goiters.[35] As a result, FNA specimens obtained from such lesions may yield detectable levels of FNA-CT, underscoring the importance of having a cutoff level for this test to prevent misinterpretation of samples. FNA-CT may also be falsely elevated, particularly in patients with high serum CT levels, due to potential peripheral blood contamination of the needle washout fluid. However, in this study, it was not believed that high FNA-CT levels in nonmedullary nodules were the result of peripheral blood contamination, because the serum CT levels in these patients were not notably high (Figure 1). Kudo et al.[28] evaluated five MTC patients with FNA-CT levels ranging from 17,000 to 560,000 pg/mL. Their findings demonstrated that FNA-CT values should exhibit a significant increase in cases of MTC, regardless of the cytology results. This study revealed that FNA-CT levels consistently exhibited elevation in all instances of MTC (Figure 3) despite the wide range of Bethesda classifications in cytologic evaluations. Although the number of patients within the cytologic subgroups is somewhat limited, this reinforces the substantial advantage of high FNA-CT values over cytology.

Although the median FNA-CT value in our study was 200,000 pg/mL, there was also a patient with MTC whose FNA-CT was as low as 431 pg/mL. Furthermore, some nodules with nonmedullary pathology had FNA-CT values as high as 8873 pg/mL (Figure 1). Previously, the threshold established for nonmedullary thyroid nodules[36] was deemed more appropriate for clinical use. However, this comprehensive study underlines that clinics need to set cutoff values.

Most patients diagnosed using the new method at our clinic had micro-MTC, in contrast to the past, where 90% of diagnoses were macro-MTC. Additionally, the median CT level in group 2 was 994 pg/mL, whereas it was 57.8 pg/mL in group 1. It is important to note that CT levels are correlated with the tumor burden.[37] Micro-MTC exhibits aggressive features like multifocality and distant metastasis, similar to macro-MTC.[38] However, patients with micro-MTC experience fewer extrathyroidal extension and cervical lymph node metastasis than macro-MTC patients. Managing micro-MTC, which generally presents with a relatively low disease burden, should be viewed as an opportunity to improve the prognosis. Detecting MTC significantly impacts a patient's outcome, as delays in diagnosis and incomplete initial treatment are associated with a poorer prognosis.[2,16,39]

CT-negative micro-MTCs were incidentally found in four patients who underwent thyroidectomy at our hospital. In a study with a similar rate and size to ours, Chambon et al.[40] reported two CT-negative micro-MTCs (sized 1 and 4 mm, respectively) among 2733 thyroidectomy patients. Zhou et al.[41] assessed 19 CT-negative MTC cases among 158 patients with MTC, concluding that this type of tumor was smaller and more differentiated. Recently, Licata et al.[42] conducted a literature review and identified 47 cases of CT-negative MTCs with varying behavior, spanning from nonaggressive to aggressive. Although CT-negative MTCs are generally categorized as low-risk because they tend to be small, it is essential to consider that they may exhibit heterogeneous courses, even if very rarely. Despite CT screening, the potential for CT-negative MTC should always be considered.

As a limitation of this study, we used TI-RADS[17] to select suspicious nodules for FNA in those smaller than 1 cm. Most classification systems, including TI-RADS, are primarily designed for papillary thyroid carcinoma. Research on the application of TI-RADS for assessing MTC is limited,[43,44] and its applicability to MTC patients remains insufficient. Consequently, this study might have missed some patients with micro-MTC. Additionally, because not all patients who underwent FNA-CY and FNA-CT proceeded to surgery, the specificity of FNA-CY and FNA-CT could not be calculated with high reliability.

Various other tests can be employed in the diagnosis of MTC. Immunocytochemistry (ICC) for CT has thus far been the sole tool used to enhance the diagnostic precision of FNA-CY for detecting MTC.[45] Given that MTC can closely resemble other conditions, ICC for CT may reveal a fraction of MTC cases, particularly with unusual morphology, provided that knowledge of elevated CT serum levels exists. The feasibility of this approach is restricted to situations where there is a strong suspicion of MTC and when cytologic samples have ample cellularity. In cases of inadequate or suboptimal cytologic specimens, identifying MTC may not be possible using this method. Recently, the introduction of molecular testing kits to confirm MTC diagnoses using inconclusive FNA samples has marked a substantial advancement.[46–49] Nevertheless, it is essential to highlight the lack of comprehensive prospective studies that specifically investigate the assessment and screening of MTC through these molecular testing kits. The persistent challenges related to accessibility and cost are worth noting, particularly for countries outside the United States.[50]

In conclusion, early detection of more cases of MTC has become feasible through the routine measurement of serum CT in all patients with nodular thyroid disease and the performance of FNA-CT in those with elevated levels. It is advisable to consider routine serum CT measurements for all patients with nodular thyroid disease to facilitate the early diagnosis of sporadic MTC. Furthermore, patients with thyroid nodules, regardless of size and elevated CT levels, should undergo FNA-CT to rule out an MTC diagnosis. With robust statistical evidence, this study demonstrated that the sensitivity of FNA-CT and serum CT is significantly superior to that of FNA-CY. In

contrast to arbitrary choices in other studies, the variation in cutoff values discovered in this study emphasizes the importance of each institution determining its cutoff value. However, further extensive case analyses are essential for establishing a universal FNA-CT cutoff value. Additionally, there is a need for updated guidelines regarding serum CT, FNA-CT, and their respective cutoff levels.

AUTHOR CONTRIBUTIONS

Berna Evranos Ogmen: Investigation, methodology, data curation, resources, conceptualization, software, writing–original draft, and article review and editing. **Nurcan Ince**: Methodology, investigation, data curation, and conceptualization. **Aysegul Aksoy Altınboga**: Methodology and investigation. **Leyla Akdogan**: Investigation and data curation. **Sefika Burcak Polat**: Investigation and validation. **Birgul Genc**: Software and formal analysis. **Ebru Menekse**: Conceptualization, methodology, and validation. **Cevdet Aydin**: Supervision. **Oya Topaloglu**: Supervision. **Reyhan Ersoy**: Supervision. **Bekir Cakir**: Supervision, conceptualization, and visualization.

ACKNOWLEDGMENTS

This research received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors. No funds, grants, or other support was received. This study was performed in line with the principles of the Declaration of Helsinki. Approval was granted by the Ethics Committee of Ankara Bilkent City Hospital (date: 02/2019; no.: E1-21-1898). Informed consent was obtained from all individual participants included in the study.

CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

ORCID

Berna Evranos Ogmen https://orcid.org/0000-0002-1848-888X
Aysegul Aksoy Altınboga https://orcid.org/0000-0003-1484-7619

REFERENCES

1. Fagin JA, Wells SA Jr. Biologic and clinical perspectives on thyroid cancer. *N Engl J Med*. 2016;375(11):1054-1067. doi:10.1056/nejmra1501993
2. Trimboli P, Giovanella L, Crescenzi A, et al. Medullary thyroid cancer diagnosis: an appraisal. *Head Neck*. 2014;36(8):1216-1223. doi:10.1002/hed.23449
3. Batista RL, Toscanini AC, Brandao LG, Cunha-Neto MB. False positive results using calcitonin as a screening method for medullary thyroid carcinoma. *Indian J Endocrinol Metab*. 2013;17(3):524-528. doi:10.4103/2230-8210.111677
4. Haugen BR, Alexander EK, Bible KC, et al. 2015 American Thyroid Association management guidelines for adult patients with thyroid nodules and differentiated thyroid cancer: The American Thyroid Association Guidelines Task Force on Thyroid Nodules and Differentiated Thyroid Cancer. *Thyroid*. 2016;26(1):1-133. doi:10.1089/thy.2015.0020
5. Gharib H, Papini E, Garber JR, et al. American Association of Clinical Endocrinologists, American College of Endocrinology, and Associazione Medici Endocrinologi medical guidelines for clinical practice for the diagnosis and management of thyroid nodules--2016 update. *Endocr Pract*. 2016;22(5):622-639.
6. Trimboli P, Treglia G, Guidobaldi L, et al. Detection rate of FNA cytology in medullary thyroid carcinoma: a meta-analysis. *Clin Endocrinol*. 2015;82(2):280-285. doi:10.1111/cen.12563
7. Pusztaszeri MP, Bongiovanni M, Faquin WC. Update on the cytologic and molecular features of medullary thyroid carcinoma. *Adv Anat Pathol*. 2014;21(1):26-35. doi:10.1097/pap.0000000000000004
8. Trimboli P, Giannelli J, Marques B, Piccardo A, Crescenzi A, Deandrea M. Head-to-head comparison of FNA cytology vs. calcitonin measurement in FNA washout fluids (FNA-CT) to diagnose medullary thyroid carcinoma. A systematic review and meta-analysis. *Endocrine*. 2022;75(1):33-39. doi:10.1007/s12020-021-02892-x
9. Wells SA Jr, Asa SL, Dralle H, et al. American Thyroid Association Guidelines Task Force on Medullary Thyroid C. Revised American Thyroid Association guidelines for the management of medullary thyroid carcinoma. *Thyroid*. 2015;25(6):567-610. doi:10.1089/thy.2014.0335
10. Lei R, Wang Z, Qian L. Ultrasonic characteristics of medullary thyroid carcinoma: differential from papillary thyroid carcinoma and benign thyroid nodule. *Ultrasound Q*. 2021;37(4):329-335. doi:10.1097/ruq.0000000000000508
11. Russell MD, Orloff LA. Ultrasonography of the thyroid, parathyroids, and beyond. *HNO*. 2022;70(5):333-344. doi:10.1007/s00106-022-01162-0
12. Kim SH, Kim BS, Jung SL, et al. Ultrasonographic findings of medullary thyroid carcinoma: a comparison with papillary thyroid carcinoma. *Korean J Radiol*. 2009;10(2):101-105. doi:10.3348/kjr.2009.10.2.101
13. Trimboli P, Nasrollah N, Amendola S, et al. Should we use ultrasound features associated with papillary thyroid cancer in diagnosing medullary thyroid cancer? *Endocr J*. 2012;59(6):503-508. doi:10.1507/endocrj.ej12-0050
14. Fukushima M, Ito Y, Hirokawa M, et al. Excellent prognosis of patients with nonhereditary medullary thyroid carcinoma with ultrasonographic findings of follicular tumor or benign nodule. *World J Surg*. 2009;33(5):963-968. doi:10.1007/s00268-009-9939-z
15. Trimboli P, Giovanella L, Valabrega S, et al. Ultrasound features of medullary thyroid carcinoma correlate with cancer aggressiveness: a retrospective multicenter study. *J Exp Clin Cancer Res*. 2014;33(1):87. doi:10.1186/s13046-014-0087-4
16. Gharib H, Papini E, Paschke R, et al. American Association of Clinical Endocrinologists, Associazione Medici Endocrinologi, and European Thyroid Association Medical guidelines for clinical practice for the diagnosis and management of thyroid nodules: executive summary of recommendations. *Endocr Pract*. 2010;16(3):468-475. doi:10.4158/ep.16.3.468
17. Russ G, Bonnema SJ, Erdogan MF, Durante C, Ngu R, Leenhardt L. European Thyroid Association guidelines for ultrasound malignancy risk stratification of thyroid nodules in adults: the EU-TIRADS. *Eur Thyroid J*. 2017;6(5):225-237. doi:10.1159/000478927
18. Cibas ES, Ali SZ. The 2017 Bethesda System for reporting thyroid cytopathology. *Thyroid*. 2017;27(11):1341-1346. doi:10.1089/thy.2017.0500
19. Cibas ES, Ali SZ; NCI Thyroid FNA State of the Science Conference. The Bethesda System for reporting thyroid cytopathology. *Am J Clin Pathol*. 2009;132(5):658-665. doi:10.1309/ajcpphlwmi3jv4la
20. Elisei R, Bottici V, Luchetti F, et al. Impact of routine measurement of serum calcitonin on the diagnosis and outcome of medullary thyroid cancer: experience in 10,864 patients with nodular thyroid disorders. *J Clin Endocrinol Metab*. 2004;89(1):163-168. doi:10.1210/jc.2003-030550
21. Vardarli I, Weber M, Weidemann F, Fuhrer D, Herrmann K, Gorges R. Diagnostic accuracy of routine calcitonin measurement for the detection of medullary thyroid carcinoma in the management of patients with nodular thyroid disease: a meta-analysis. *Endocr Connect*. 2021;10(3):358-370. doi:10.1530/ec-21-0030

22. Liu CY, Bychkov A, Agarwal S, et al. Cytologic diagnosis of medullary thyroid carcinoma in the Asia-Pacific region. *Diagn Cytopathol*. 2021;49(1):60-69. doi:10.1002/dc.24586
23. Dyhdalo KS, Chute DJ. Barriers to the recognition of medullary thyroid carcinoma on FNA: Implications relevant to the new American Thyroid Association guidelines. *Cancer Cytopathol*. 2018;126(6):397-405. doi:10.1002/cncy.21990
24. Yang GC, Fried K, Levine PH. Detection of medullary thyroid microcarcinoma using ultrasound-guided fine needle aspiration cytology. *Cytopathology*. 2013;24(2):92-98. doi:10.1111/j.1365-2303.2012.00969.x
25. Liu Z, Zhou W, Han R, et al. Cytology versus calcitonin assay in fine-needle aspiration biopsy wash-out fluid (FNAB-CT) in diagnosis of medullary thyroid microcarcinoma. *Endocrine*. 2021;74(2):340-348. doi:10.1007/s12020-021-02759-1
26. Marques B, Cunha N, Martins RG, et al. Lymph node metastases of medullary thyroid cancer: role of calcitonin in the washout fluid of fine-needle aspiration. *Int J Endocrinol*. 2020;2020:9267972--9267976. doi:10.1155/2020/9267972
27. Boi F, Maurelli I, Pinna G, et al. Calcitonin measurement in wash-out fluid from fine needle aspiration of neck masses in patients with primary and metastatic medullary thyroid carcinoma. *J Clin Endocrinol Metab*. 2007;92(6):2115-2118. doi:10.1210/jc.2007-0326
28. Kudo T, Miyauchi A, Ito Y, Takamura Y, Amino N, Hirokawa M. Diagnosis of medullary thyroid carcinoma by calcitonin measurement in fine-needle aspiration biopsy specimens. *Thyroid*. 2007;17(7):635-638. doi:10.1089/thy.2006.0338
29. Diazzi C, Madeo B, Taliani E, et al. The diagnostic value of calcitonin measurement in wash-out fluid from fine-needle aspiration of thyroid nodules in the diagnosis of medullary thyroid cancer. *Endocr Pract*. 2013;19(5):769-779. doi:10.4158/ep12420.or
30. Trimboli P, Cremonini N, Ceriani L, et al. Calcitonin measurement in aspiration needle washout fluids has higher sensitivity than cytology in detecting medullary thyroid cancer: a retrospective multicentre study. *Clin Endocrinol*. 2014;80(1):135-140. doi:10.1111/cen.12234
31. de Crea C, Raffaelli M, Maccora D, et al. Calcitonin measurement in fine-needle aspirate washouts vs. cytologic examination for diagnosis of primary or metastatic medullary thyroid carcinoma. *Acta Otorhinolaryngol Ital*. 2014;34(6):399-405.
32. Trimboli P, Nigri G, Romanelli F, et al. Medullary thyroid nodules by measurement of calcitonin (Ct) in aspiration needle washout in patients with multinodular goiter and moderately elevated serum Ct. *Exp Clin Endocrinol Diabetes*. 2012;120(4):234-237. doi:10.1055/s-0031-1291344
33. Trimboli P, Rossi F, Baldelli R, et al. Measuring calcitonin in washout of the needle in patients undergoing fine needle aspiration with suspicious medullary thyroid cancer. *Diagn Cytopathol*. 2012;40(5):394-398. doi:10.1002/dc.21731
34. Trimboli P, D'Aurizio F, Tozzoli R, Giovanella L. Measurement of thyroglobulin, calcitonin, and PTH in FNA washout fluids. *Clin Chem Lab Med*. 2017;55(7):914-925. doi:10.1515/cclm-2016-0543
35. Zayed AA, Ali MK, Jaber OI, et al. Is Hashimoto's thyroiditis a risk factor for medullary thyroid carcinoma? Our experience and a literature review. *Endocrine*. 2015;48(2):629-636. doi:10.1007/s12020-014-0363-2
36. Giovanella L, Ceriani L, Bongiovanni M. Calcitonin measurement on fine needle washouts: preanalytical issues and normal reference values. *Diagn Cytopathol*. 2013;41(3):226-229. doi:10.1002/dc.22804
37. Park H, Park J, Choi MS, et al. Preoperative serum calcitonin and its correlation with extent of lymph node metastasis in medullary thyroid carcinoma. *Cancers*. 2020;12(10):2894. doi:10.3390/cancers12102894
38. Kim JH, Pyo JS, Cho WJ. Clinicopathological significance and prognosis of medullary thyroid microcarcinoma: a meta-analysis. *World J Surg*. 2017;41(10):2551-2558. doi:10.1007/s00268-017-4031-6
39. Pacini F, Castagna MG, Cipri C, Schlumberger M. Medullary thyroid carcinoma. *Clin Oncol*. 2010;22(6):475-485. doi:10.1016/j.clon.2010.05.002
40. Chambon G, Alovisetti C, Idoux-Louche C, et al. The use of preoperative routine measurement of basal serum thyrocalcitonin in candidates for thyroidectomy due to nodular thyroid disorders: results from 2733 consecutive patients. *J Clin Endocrinol Metab*. 2011;96(1):75-81. doi:10.1210/jc.2010-0162
41. Zhou Q, Yue S, Cheng Y, Jin J, Xu H. Clinical and pathological analysis of 19 cases of medullary thyroid carcinoma without an increase in calcitonin. *Exp Toxicol Pathol*. 2017;69(8):575-579. doi:10.1016/j.etp.2017.05.003
42. Licata L, Di Fidio CA, Vacante M, et al. A rare case of negative serum calcitonin in metastatic medullary thyroid carcinoma: diagnosis, treatment, and follow-up strategy. *Am J Case Rep*. 2022;23:e935207. doi:10.12659/ajcr.935207
43. Zhao J, Zheng X, Gao M, et al. Ultrasound features of medullary thyroid cancer as predictors of biological behavior. *Cancer Imag*. 2021;21(1):33. doi:10.1186/s40644-021-00402-w
44. Zhu J, Li X, Wei X, et al. The application value of modified thyroid imaging report and data system in diagnosing medullary thyroid carcinoma. *Cancer Med*. 2019;8(7):3389-3400. doi:10.1002/cam4.2217
45. Trimboli P, Guidobaldi L, Bongiovanni M, Crescenzi A, Alevizaki M, Giovanella L. Use of fine-needle aspirate calcitonin to detect medullary thyroid carcinoma: a systematic review. *Diagn Cytopathol*. 2016;44(1):45-51. doi:10.1002/dc.23375
46. Lukyanov SA, Sergiyko SV, Titov SE, et al. New opportunities for preoperative diagnosis of medullary thyroid carcinoma. *Biomedicines*. 2023;11(5):1473. doi:10.3390/biomedicines11051473
47. Randolph GW, Sosa JA, Hao Y, et al. Preoperative identification of medullary thyroid carcinoma (MTC): clinical validation of the Afirma MTC RNA-sequencing classifier. *Thyroid*. 2022;32(9):1069-1076. doi:10.1089/thy.2022.0189
48. Hao Y, Choi Y, Babiarz JE, et al. Analytical verification performance of Afirma genomic sequencing classifier in the diagnosis of cytologically indeterminate thyroid nodules. *Front Endocrinol*. 2019;10:438. doi:10.3389/fendo.2019.00438
49. Kloos RT, Monroe RJ, Traweek ST, Lanman RB, Kennedy GC. A genomic alternative to identify medullary thyroid cancer preoperatively in thyroid nodules with indeterminate cytology. *Thyroid*. 2016;26(6):785-793. doi:10.1089/thy.2016.0001
50. Wu JX, Lam R, Levin M, Rao J, Sullivan PS, Yeh MW. Effect of malignancy rates on cost-effectiveness of routine gene expression classifier testing for indeterminate thyroid nodules. *Surgery*. 2016;159(1):118-126. doi:10.1016/j.surg.2015.05.035

How to cite this article: Ogmen BE, Ince N, Aksoy Altınboga A, et al. An old friend, a new insight: calcitonin measurement in serum and aspiration needle washout fluids significantly increases the early and accurate detection of medullary thyroid cancer. *Cancer Cytopathol*. 2023;1-8. doi:10.1002/cncy.22779