Inappropriate Requests for Serum Calcitonin Testing: Insights from a Retrospective Analysis

Solicitações Inadequadas de Testes de Calcitonina Sérica: Perspectivas de uma Análise Retrospectiva

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Abstract

Early diagnosis influences the prognosis of medullary thyroid carcinoma (MTC). Serum calcitonin measurement before thyroidectomy may contribute to its diagnosis. However, calcitonin testing should be ordered appropriately to avoid unnecessary testing. This study aimed to evaluate the appropriateness of requests for calcitonin testing at a tertiary hospital. We retrospectively evaluated calcitonin measurements performed between 2008 and 2017. We compared cases with an appropriate justification with those in which the justification was inappropriate or unreported, cases with and without detectable calcitonin levels, and cases with and without MTC. A total of 1,453 calcitonin measurements were performed in 915 patients. The most frequent indication for testing was the presence of a thyroid nodule. The indication was considered appropriate in 772 cases (84.4%) and inappropriate or unreported in 143 cases (15.6%). Most cases had undetectable calcitonin levels (n = 757; 82.7%). A higher proportion of cases with an inappropriate or unreported justification had detectable calcitonin levels (30.1% versus 14.9%). Among cases with detectable calcitonin levels, concentrations ranged from 2.00 to 527.00 pg/mL. Nineteen patients (2.1%) received a final diagnosis of MTC, most of whom (n = 14; 73.7%) had detectable calcitonin levels. Among these 14 patients, eight (57.1%) had concentrations >100 pg/mL. In conclusion, a considerable proportion of requests for serum calcitonin testing were inadequately justified or lacked justification. In these cases and among patients with MTC, a higher prevalence of detectable calcitonin levels was observed. This study highlights the need to establish specific protocols for ordering calcitonin testing to minimize the negative impact of unnecessary investigations and false-positive results.

Keywords: Calcitonin; Carcinoma, Medullary; Diagnosis; Diagnostic Techniques and Procedures.

Resumo

O diagnóstico precoce influencia o prognóstico do carcinoma medular da tireoide (CMT). A dosagem de calcitonina sérica antes da tireoidectomia pode contribuir para esse diagnóstico. No entanto, o exame deve ser solicitado de forma adequada, a fim de evitar solicitações desnecessárias. O objetivo deste estudo foi avaliar a adequação das solicitações de dosagem de calcitonina em um hospital terciário. Avaliamos retrospectivamente as dosagens de calcitonina realizadas entre 2008 e 2017. Comparamos os casos com justificativa adequada com aqueles cuja justificativa foi inadequada ou não informada, os casos com e sem níveis detectáveis de calcitonina e os casos com e sem CMT. Foram realizadas 1.453 dosagens de calcitonina em 915 pacientes. A indicação mais frequente para a solicitação do exame foi a presença de nódulo tireoidiano. A justificativa para a solicitação foi considerada adequada em 772 casos (84,4%) e inadequada ou não informada em 143 casos (15,6%). A maioria dos casos apresentou níveis indetectáveis de calcitonina (n = 757; 82,7%). Uma proporção maior de casos com justificativa inadequada ou não informada apresentou níveis detectáveis de calcitonina (30,1% versus 14,9%). Nos casos com níveis detectáveis de calcitonina, as concentrações variaram entre 2,00 e 527,00 pg/mL. Dezenove pacientes (2,1%) receberam diagnóstico final de CMT, sendo que a maioria deles (n = 14; 73,7%) apresentou níveis detectáveis de calcitonina. Entre esses 14 pacientes, oito (57,1%) apresentaram concentrações >100 pg/mL. Em conclusão, uma proporção considerável das solicitações de dosagem de calcitonina sérica foi inadequadamente justificada ou não apresentou justificativa. Nesses casos e entre os pacientes com CMT, observou-se maior prevalência de níveis detectáveis de calcitonina. Este estudo ressalta a necessidade de estabelecer protocolos específicos para a solicitação do exame, a fim de minimizar o impacto negativo de investigações desnecessárias e de resultados falso-positivos.

Palavras-chave: Calcitonina. Carcinoma Medular. Diagnóstico. Técnicas e Procedimentos Diagnósticos.

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INTRODUCTION

Medullary thyroid carcinoma (MTC), a neuroendocrine tumor from thyroid parafollicular cells, accounts for 3–4% of thyroid cancers and is often aggressive, frequently presenting with nodal metastases; thus, early diagnosis is critical for prognosis.[1-3]

Despite the potential severity and relevance of early diagnosis, due to its rarity, MTC is not often considered as a diagnostic possibility in clinical practice. In addition, the main test used to investigate thyroid nodules, fine needle aspiration (FNA), has lower sensitivity for MTC than to other thyroid tumours such as differentiated thyroid carcinomas.[4] Thus, MTC diagnosis can be quite delayed, resulting in poorer prognosis. Therefore, methods that can detect the primary tumour, as well as possible recurrences, are highly desirable. In this sense, the parafollicular cells produce proteins, such as carcinoembryonic antigen (CEA) and, particularly, calcitonin, whose serum concentrations can be used as markers for both diagnosis and follow-up of affected patients.[5]

Although serum calcitonin measurement can be very useful in the initial etiological investigation of thyroid nodules in general, providing early diagnosis of MTC,[6] its routine use for evaluating these lesions remains controversial. The European Thyroid Association[1] recommends routine measurement of the hormone in evaluating patients with a thyroid nodule, whereas the recommendations by the Thyroid Department of the Brazilian Society of Endocrinology and Metabolism[2] do not, and American guidelines[3] leave the decision to the attending physician.

This lack of consensus is due to low MTC prevalence, false positives, risks from surgical treatment after inadequate interpretation, and cost. In fact, despite their sensitivity, calcitonin assays are subject to failure, plus the hormone is not specific for MTC and there may be several differential diagnoses for hypercalcitoninemia. False-positive and false-negative results have been reported in various conditions and diseases, such as Hashimoto's thyroiditis, other gland cancers or pancreatic neuroendocrine neoplasms, or even due to failures in dosage from interference by rheumatoid factor or heterophile antibodies.[7-10] Another problem with calcitonin measurement is that the cut-off value for differentiating between benign and malignant lesions has not yet been fully established. In general, serum concentrations >100 pg/mL would be considered very suggestive for MTC diagnosis and those <10 pg/mL would indicate a lower probability for the tumour. Levels between 10 and 100 pg/mL would be considered inconclusive, with some authors considering a stimulus test, currently performed using calcium gluconate.[11] Another factor influencing the decision to request calcitonin measurements in thyroid nodular evaluation is cost. However, some authors have reported that it is cost-effective compared to other routinely requested screening tests in certain patient groups.[12]

Despite the difficulties measuring the hormone and the lack of consensus for its use in evaluating all thyroid nodules, it is undeniable that the test helps in earlier diagnosis of MTC and approach in the less advanced stages, being associated with higher cure and survival rates.[13,14] In addition to its role in patient diagnosis and prognosis, measurement of serum calcitonin is also essential in monitoring MTC. Undetectable concentrations indicate absence, while progressive elevation indicates residual disease or relapse.[15] On the other hand, disproportionately low concentrations in the presence of elevated CEA suggest poorly differentiated MTC.[3] Considering the diagnostic, prognostic and follow-up relevance for patients with MTC, as opposed to the limitations cited for the test, it is extremely important that calcitonin measurement be requested sparingly and analyzed by professionals who are able to assess the impact of the results presented. Thus, the aim of this study was to assess the adequacy for calcitonin test requests in a tertiary hospital.

MATERIALS AND METHODS

Study design

This is an observational, retrospective study conducted using indirect data and reported according to Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) guidelines.

Study hospital

This study was carried out in the Hospital das Clínicas - Botucatu Medical School - Sao Paulo State University – Unesp, a tertiary hospital specialized in the treatment of thyroid cancer patients. It is a public university hospital with 415 beds, 294 consultation rooms and 35 medical specialties located in the interior of São Paulo State, Brazil. The hospital serves an area with a population of approximately 3 million people, with an average of 2 million tests, 600,000 consultations, 20,000 admissions and 11,000 operations per year. In this hospital, three groups of students, 90 each from the 4th

to the 6th year of medical school internship, 317 specialist physicians and 476 residents of various medical specialties, in addition to approximately 254 university professors, carry out activities with patients. Routine serum calcitonin measurement became available at this hospital in 2008 and is freely accessible to all physicians and medical intern students. The hospital endocrinology service requests serum calcitonin measurement in the following situations: appearance of suspicious nodules on ultrasound (US), undetermined cytological diagnosis or suspected/presence of C cells, family history or clinical suspicion of MTC or Multiple Endocrine Neoplasia Type 2 (MEN2), and indication for thyroidectomy. So far, the number of requested tests has not been quantified, nor has there been an assessment on the suitability of indications for requesting the test.

Study sample

Laboratory and medical records were analyzed for patients who underwent serum calcitonin testing, evaluating all results performed in the hospital's central laboratory between 2008 and 2017. This was a convenience sample, and cases were included irrespective of the test indication or the treatment administered.

Variables of interest and outcomes

The main variable of interest was serum calcitonin level and the main outcome evaluated was appropriateness of the test request. We initially quantified the number of calcitonin measurements and number of patients submitted to the test; hormone concentrations were also evaluated. The sample description included data on case diagnosis, gender, age, and self-reported skin color. Skin color was classified as White or non-White, based on the categories established by the Brazilian Institute of Geography and Statistics (IBGE): White, Black, Brown (Pardo), Yellow, and Indigenous.[16] The cases where calcitonin test request was considered adequate or inadequate/not given were compared with final diagnosis of MTC. Requests due to diagnosis or family history of MTC and NEM2 were considered adequate. In addition, due to the lack of consensus regarding calcitonin levels in thyroid nodule evaluation and the implications of a lack of correct preoperative MTC diagnosis, requests due to thyroid nodule, indication for thyroidectomy and other conditions that could imply diagnostic doubt or be associated with NEM2 (for example pheochromocytoma and hyperparathyroidism) were also considered appropriate. Requests due to other reasons were considered inappropriate and where no reason was given on the test request form or in the patient's medical record, requests were considered not given.

Cases with detectable or undetectable calcitonin, as described below, were also compared, and those with and without MTC, according to anatomopathological diagnosis,[17] with regard to epidemiological data, justification for collection, final diagnosis and calcitonin concentrations.

Finally, we quantified and compared the cost of adequately justified and tests with inadequate or not given justifications performed at the hospital central laboratory and based on the June 2018 local hormone test price calculated in American dollars (USD).

Measurement methodology

Chemiluminescence immunometric solid-phase immunoassay was used to quantify serum calcitonin in human serum or plasma. Manufacturer reference values were <18.2 pg/mL for men and <11.5 pg/mL for woman (Siemens, LABPACK, United Kingdom). Calcitonin was considered undetectable at <2.0 pg/mL and detectable at >2.0 pg/mL. On-going and categorized calcitonin evaluations were performed, with ranges of <10 pg/mL, 10-20 pg/mL, 20-100 pg/mL and >100 pg/mL. In cases with more than one test result, the highest value was considered.

Ethical aspects

This study was carried out after approval from Botucatu Medical School Research Ethics Committee (Certificate of Presentation of Ethical Appreciation number-no. 81951617.6.0000.5411, protocol no. 2.497.576), in line with National Health Council Resolution no. 466/2012.

Statistical methods

Collected data were tabulated in an Excel® spreadsheet (Microsoft Corporation, United States) and submitted to statistical analysis using Statistical Analysis System- SAS Version 9.4 and R Version 3.0. Descriptive statistical data analysis was performed, with frequency and percentages for categorized variables, and mean, median, standard deviation, minimum and maximum values for quantitative variables. The chi-square test was used to verify association between groups classified according to calcitonin detectability and variables such as gender, self-reported skin color, final MTC diagnosis, and hormone range. It was also used to compare patients with or without MTC in terms of gender,

self-reported skin color and calcitonin range. The proportion test was used to compare the proportion of appropriate and inappropriate/not given collections. The Student's t-test was used to evaluate continuous variables, concerning the outcomes, in the case of variables that presented symmetrical distribution in the normality test. In contrast, a generalized linear model with gamma distribution was adjusted for variables with asymmetrical distribution. Significance level was considered as $p < 0.05$.

RESULTS

A total of 1453 calcitonin tests were performed in a total of 915 patients with 538 exams consisting of repetitions performed during follow-up of patients with MTC/MEN2. Patient mean age was 53.9 years, with a majority of women (n=765; 83.6%) and self-reported White skin color (n=831; 94.6%). The lowest detectable calcitonin value was 2.00 pg/mL and the highest was 527.00 pg/mL (median = 4.1 pg/mL). One patient had a value above the detectable value (>2000 pg/mL). Most patients had undetectable calcitonin (n=757; 82.7%). The most frequent justification for calcitonin collection was thyroid nodule (n=676; 73.9%). In 27 patients (2,9%), the justification for collecting the exam was the confirmed or suspected diagnosis of MTC or family history of the neoplasm. Reason for test request was not given in 116 patients (12.9%). Nineteen cases (2.1%) had a confirmed final tumour diagnosis (Table 1).

Considering cases according to calcitonin detectability (Table 1), most were undetectable (n=757, 82.7%; $p < 0.0001$) with higher percentages of females (n=679; 89.7%; $p < 0.0001$). Higher percentages of self-reported White skin color were observed in both groups, and the main reason for requesting the test was a thyroid nodule (n=86 - 54.5% and n=590 - 77.9%, respectively). A minority had a final diagnosis of MTC (n=19; 2,1%). However, most of the patients with MTC (n=14; 73.7%) had detectable calcitonin. In the detectable group, 123 patients (77.8%) had calcitonin values <10 pg/mL and 12 had calcitonin values >100 pg/mL (7.6%). Groups did not differ for the other evaluated parameters (Table 1).

Comparing patients with and without MTC (Table 2), as expected, those without were more numerous ($p < 0.0001$). The frequency of women was higher in the without MTC group (n=753; 84.0%; p= 0.015). In patients without MTC, the main reason for requesting the test was thyroid nodule (n= 672; 75.0%). In cases with MTC, the most frequent justifications were clinical suspicion or already established tumour diagnosis (n=15; 79.0%) and thyroid nodule (n=4; 21.0%). In 676 cases where calcitonin measurement was requested due to the presence of a thyroid nodule, four (0.6%) had a final diagnosis of MTC. Calcitonin test results in these patients were 7.60, 9.33, 78.30 and >2,000.00 pg/mL. The MTC group had higher calcitonin concentrations ($p < 0.0001$). As for calcitonin ranges, higher percentages of patients without MTC (n=872 - 97.3% versus-vs n=8 - 42.1%) had hormone concentrations below 10 pg/mL, while higher percentages of cases with MTC (n=8 - 42.1% vs n=3 - 0.3%) showed calcitonin >100 pg/mL % ($p < 0.0001$). As for the three cases without MTC but with calcitonin values >100 pg/mL (0.3%), the first was a laboratory error, the second had several confounding factors and did not have a thyroid nodule, and the third had other types of neoplasia (hepatic carcinoid and colon cancer) and, despite MTC being suspected, diagnosis was not confirmed. In the case of the laboratory error, the patient had no evidence of thyroid disease and, unfortunately, the cause of the error could not be determined. No difference was observed between groups for the other evaluated parameters (Table 2).

Table 1

Description of patients in general and according to the hormone detectable or not.

Parameter	Calcitonin			p-value‡
	Total	Detectable	Undetectable	
Cases#	915 (100)	158 (17,3)	757 (82,7)	<0.0001¶
Age (years)*	53.93 ± 17.90	52.94 ± 19.58	54.14 ± 17.53	0.4450†
Female#	765 (83,6)	86 (54,4)	679 (89,7)	<0.0001¤
Self-reported White skin color #	831 (94,6)	139 (93,3)	692 (94,9)	0.1635¤
Justification for the exam:				
Thyroid nodule#	676 (73.9)	86 (54.5)	590 (77.9)	
Not informed#	116 (12.9)	34 (21.5)	82 (10.8)	Comparison not possible
Medullary thyroid cancer (confirmed or suspected diagnosis) #	15 (1.6)	10 (6.3)	5 (0.7)	
Family history of medullary thyroid cancer#	12 (1.3)	2 (1.3)	10 (1.3)	
Final diagnosis of medullary thyroid câncer#	19 (2.1)	14 (8.9)	5 (0.7)	<0.0001¤
Categorized calcitonin:				
< 10 pg/mL#	880 (96.2)	123 (77.8)	757 (100.0)	
10-20 pg/mL#	14 (1.5)	14 (8.9)	0	<0.0001¤
20- 100 pg/mL#	9 (1.0)	9 (5.7)	0	
> 100 pg/mL#	12 (1.3)	12 (7.6)	0	
Calcitonin (pg/mL)**	4.1 (2.0; 527.0)	4.1 (2.0; 527.0)	-	-

† Comparison between cases with detectable and undetectable calcitonin. ¶ Proportions test. #Number (% - percentage). *Average± standard deviation. **Median (minimum; maximum). † Student´s test. ¤ Chi-squared test. p-value < 0.05 indicates statistical significance. pg/mL: picograms per milliliter.

Table 2

Description of patients with or without the final diagnosis of medullary thyroid carcinoma.

Parameter	Medullary thyroid carcinoma		p-value╬
	Without	With	
Cases#	896 (98.0)	19 (2.0)	<0.0001¶
Age (years)*	54.00 ± 17.95	50.97 ± 15.51	0,4663†
Female#	753 (84.0)	12 (63.2)	0.0150¤
Self-reported White skin color #	813 (94.5)	18 (100.0)	0.7917¤
Justification for the exam			
Thyroid nodule#	672 (75.0)	4 (21.0)	
Not informed#	116 (13.0)	0	Comparison not possible
Medullary thyroid cancer (confirmed or suspected diagnosis) #	0	15 (79.0)	
Family history of medullary thyroid cancer#	12 (1.3)	0	
Calcitonin (pg/mL)**	3.7 (1.28; 454.0)	50.8 (6.3; 527.0)	<0.0001¶
Categorized calcitonin			
<10 pg/mL#	872 (97.3)	8 (42.1)	
10-20 pg/mL#	14 (1.7)	0	<0.0001¤
20-100 pg/mL#	7 (0.7)	3 (15.8)	
>100 pg/mL#	3 (0.3)	8 (42.1)	

╬Comparison between cases with and without CMT. ¶ Proportions test. #Number (% - percentage).*Average ± standard deviation. † Student´s t-test. ¤ Chi-squared test. ** Median. ¶ Generalized linear model with gamma distribution. p-value < 0.05 indicates statistical significance. pg/mL: picograms per milliliter.

Comparing the cases for appropriateness of requesting a calcitonin test (Table 3), justification was considered adequate (MTC, family history of MTC, thyroid nodule, plunging multinodular goiter with surgical indication, anaplastic thyroid carcinoma, adenopathy cervical cancer, pheochromocytoma, hypercalcemia/hyperparathyroidism, tumour marker, neurofibromatosis type 1, paraganglioma) in most patients (n=772 - 84.4% vs n=143 - 15.6%; p <0.0001). Adding the 538 repetitions performed during follow-up for patients with MTC/MEN2 to the 772 cases described above, makes a total of 1310 tests with adequate justifications (90%), against 143 tests (10%) with inadequate/not given justifications. Of the latter, twenty-seven cases (18.9%) had a justification considered inadequate (polyostotic fibrotic dysplasia, decompensated heart failure, multiple endocrine neoplasia type 1, follow-up of differentiated thyroid carcinoma, hypothyroidism, insulinoma, pancreatitis, as part of the patient calcium profile), which, considering the total of 915 evaluated patients, corresponds to 2.9% of cases. Of cases with inadequate/not given justification, a higher percentage had detectable calcitonin (n=43 - 30.1% vs n=115 - 14.9%; p <0.0001). In patients with adequate justification, the lowest detectable calcitonin value was 2.00 pg/mL and the highest was 527.00 pg/mL (median = 3.9), while MTC was diagnosed in 19 cases (2.1% of all patients who had a calcitonin test). For statistical calculation purposes, undetectable concentrations greater than 2000.00 pg/mL were not included. In cases where justifications were considered inappropriate/not given, the lowest detectable calcitonin value was 2.1 pg/mL and the highest was 454.0 pg/mL (median = 4.0 pg/mL). This maximum value corresponded to a single case which, upon review, was found to be a patient with differentiated thyroid carcinoma who had undetectable hormone levels measured in another laboratory, indicating that the initial result was a measurement error. Most of the patients with calcitonin >100 ng/mL had adequate justification (n=10 - 83.3% vs n=2 - 16.7%). Of those with calcitonin between 10 and 100 pg/mL and an adequate justification for requesting the test, nine had thyroid nodules, three MTC, one pheochromocytoma and in 1 case, the test was performed as a tumour marker for investigating neoplasia with an undetermined site. In this hormonal range, the reason for requesting the test was not informed in 10 cases and the reason was pancreatitis in one case. No case with a final MTC diagnosis presented inadequate/not given justification for requesting the test (Table 3).

Table 3

Description of patients regarding the adequacy of the justification for requesting serum calcitonin measurement.

Parameter	Justification		p-value*
	Adequate	Inadequate / Not informed	
Cases#	772 (84.4)	143 (15.6)	<0.0001¶
Detectable calcitonin#	115 (14.9)	43 (30.1)	<0.0001¤
Calcitonin (pg/mL)**	3.90 (2.0; 527.0)	4.65 (2.1; 454.0)	0.2466¬
Categorized calcitonin			
<10 pg/mL#	749 (97.0)	131 (91.6)	
10-20 pg/mL#	5 (0.7)	9 (6.3)	<0.0001¤
20-100 pg/mL#	8 (1.0)	1 (0.7)	
>100 pg/mL#	10 (1.3)	2 (1.4)	
Final diagnosis of medullary thyroid cancer #	19 (2.5)	0 (0.0)	-

*Comparison between cases with adequate and with inadequate/not informed requesting. #Number (% - percentage). ** Median (minimum; maximum). ¶ Proportions test. ¤ Chi-squared test. ¬ Generalized linear model with gamma distribution. p-value < 0.05 indicates statistical significance. pg/mL: picograms per milliliter.

Each calcitonin measurement costs 2.92 USD, totalling 4,242.76 USD for the 1453 measurements performed in the period. Therefore 2,254.24 USD were spent on measurements with adequate justification, 78.84 USD with inadequate justification and 338.72 USD on patients whose reason for collection was not informed (116 cases, 12.7%). Also, 1,973.92 USD were also spent on the 676 measurements due to thyroid nodule. Considering that final diagnosis in four of these patients was MTC, and that they had detectable calcitonin, one could speculate that diagnosis cost for each of these cases was 493.48 USD. However, one of these four had a concentration >2000.0 pg/mL, another 78.3 pg/mL, and two had detectable calcitonin, but <10 pg/mL. That is, the results in two cases would not raise suspicions for MTC, whilst concentrations in the other two cases increased suspicion for MTC presence. Thus, diagnosis cost in these last two cases would be 986.96 USD.

DISCUSSION

This work studied aspects related to serum calcitonin test requests in a tertiary hospital specialized in treating thyroid cancer patients between 2008 and 2017. Although the main objective was to evaluate test request adequacy, other relevant information was obtained. During the period reviewed, 1453 calcitonin tests were performed in 915 patients with values in most tests being undetectable. Almost 84% (n=757) of 896 patients without MTC had undetectable calcitonin (<2.0 pg/mL), whilst 97% (n=872) had values <10.0 pg/mL. These percentages were higher than those reported by Hauache et alii (al.),[18] who analyzed baseline calcitonin in 1,124 normal individuals, finding levels <2 pg/mL in 68.5% (n=771) and <5 pg/mL in 87.5% (n=986) of cases. Probably, the main reason for this difference was the fact that our sample was previously selected by the comorbidities it presented. It is important to highlight that the undetectable category is more relevant when monitoring a patient with MTC postoperatively. When evaluating patients with benign diseases, a thyroid nodule, for example, falls into the same clinical significance as within the reference range.

As expected in this study, from all the patients tested for calcitonin at the service, few had a final MTC diagnosis (n=19; 2%). In fact, this tumour is rare, representing less than 10% of all thyroid neoplasms. Most of the cases with MTC (n=14; almost 74%) had detectable calcitonin, which reinforces the considerable relationship between serum calcitonin and this type of neoplasm. In fact, hormone concentrations have been associated with diagnosis, tumour dimensions and patient prognosis.[19] In this study, although patients with MTC and detectable serum calcitonin showed a wide range of hormone concentrations [from 6.3 to 527 pg/mL; one patient had hormone levels so high (>2,000 pg/mL) and could not be measured], eight (42%) had values >100 pg/mL, a concentration suggesting a high probability of MTC. On the other hand, eight (42%) of MTC patients had values <10 pg/mL, suggesting a lower probability of the tumour. However, it is important to emphasize that in many of the MTC patients, calcitonin was requested for follow-up in already operated cases and those with the disease under control. Thus, the presence or absence of disease remission must have influenced hormone concentrations. In patients without MTC, calcitonin concentrations were also quite variable (from 1.28 to 454 pg/mL), with the maximum value reaching levels compatible with metastatic MTC; this was, in fact, a laboratory error. Unfortunately, the retrospective design of the study made it impossible to determine the cause of this error. Factors such as hormone instability and its biphasic half-life, as well as the presence of interfering substances in the serum — including heterophilic antibodies and macrocalcitonin — have been associated with false results.[20] Despite the wide variation in hormone levels, median calcitonin values were undoubtedly higher in MTC patients (50.8 pg/mL vs 3.7 pg/mL). The calcitonin cut-off value for diagnosing MTC is still a problem in clinical practice. Kwon et al.[21] reported that baseline levels >65 pg/mL would indicate macroscopic MTC, while Allelein et al.[22] observed that the most accurate limits for identifying MTC would be ≥ 46 pg/mL for men and ≥ 35 pg/mL for women. However, the accuracy of these cut-off levels in female patients was considered much lower than in males.[22] In addition to its use in the diagnosis, calcitonin concentrations have been associated with MTC tumour size. In this sense, Kwon et al.[21] observed that concentrations <65 pg/mL would not exclude the presence of small-volume MTC or C-cell hyperplasia (CCH), a pre-malignant alteration. According to the German Thyroid Society,[23] the best cut-off points to differentiate micro MTC from CCH, as well as other non-specific calcitonin elevations, would be 30 pg/mL and 60 pg/mL, in women and men, respectively, using chemiluminescent immunoassay with a 0.5 pg/mL sensitivity. Thus, in Germany, thyroidectomy is recommended for patients with thyroid nodules and serum calcitonin above these values.[23] In addition to preoperative

calcitonin levels being associated with tumour size, the relationship between serum hormone concentration and nodular size >30 ng/mL/mm could be associated with a higher risk of early lymph node metastases.[24] We were unable to stratify cases with concentrations between 30 and 40 pg/mL due to an insufficient sample number in this category.

Interestingly, the percentage of MTC patients with undetectable calcitonin was not negligible (n=5; 26%). Obviously, many of these values could be related to patient follow-up in those already operated and in remission. However, it should be emphasized that calcitonin does not always represent a good tumour marker. In this study, for example, of the four MTC cases where preoperative calcitonin measurement was indicated due to the presence of a thyroid nodule, two had detectable hormone, but with values <10 pg/mL. In fact, basal calcitonin level may not present good sensitivity for diagnostic screening of MTC patients who still do not have a palpable tumour, as concentrations may be normal in the initial phase of the neoplasm.[25] Furthermore, even metastatic tumours, and even tumours with positive immunohistochemistry for calcitonin, may have undetectable serum levels of the hormone. Apparently, some MTC do not expressively secrete calcitonin, making tumour diagnosis and monitoring difficult.[26] In these cases, postoperative follow-up should include periodic imaging, as these patients may have poorly differentiated MTC and these tumours are at higher risk of disease-related death and require a more aggressive follow-up strategy. Other tumour markers could be also useful.[27] However, unfortunately, the sensitivity of CEA and chromogranin A tests, for example, are even lower than that of calcitonin, and may be within normal ranges, even in patients with advanced metastatic MTC.[28] A recent umbrella review concluded that other tools available for diagnosing MTC yield inferior results compared to calcitonin measurement.[29] Therefore, further studies are needed to better define the options for the diagnosis and follow-up of non-calcitonin-secreting MTC.[30]

Regarding the main objective of this study, most cases (n=772; 84%) presented adequate justification for requesting serum calcitonin. Obviously, this percentage was influenced by situations considered suitable for indicating the test, the most frequent being the presence of a thyroid nodule (n=676; almost 74% of cases). Although serum calcitonin has been considered more sensitive than FNA and US for diagnosing MTC, the question regarding indiscriminate calcitonin measurement in nodule evaluation is still controversial.[1-3] In this study, considering the potential aggressiveness of the tumour, the risk of late diagnosis and the usefulness of hormone values in evaluating nodular disease, even in patients without clinical or cytological suspicion of MTC,[31] suspicious nodules for neoplasms was considered as adequate justification for requesting the test. Four of these patients (0.6%) had a final diagnosis of MTC, of whom two had elevated calcitonin concentrations which alerted physicians to the diagnosis.

Despite the high percentage of properly justified requests, reasons for collection were not informed or were inappropriate in 15.6% (n=143) of the patients, such as for the investigation or follow-up of heart failure, pancreatitis, hypothyroidism, type 1 endocrine neoplasia or as an item in calcium profile assessment. It is important to highlight that, although approximately 30% (n=43) of these cases had detectable calcitonin, many of these dosages were within the normal range for the essay (<18.2 pg/mL for men and <11.5 pg/mL for woman). However, high calcitonin concentrations were found in some of these cases, reaching serum levels >100 pg/mL. None of these patients were diagnosed with MTC. It is therefore essential to emphasize that adequate laboratory investigation of patients should be preceded by detailed anamnesis and physical examination, with pertinent diagnostic hypotheses elaborated to guide test requests. It is also important to emphasize that the adequate indication for serum calcitonin measurement can result in early MTC diagnosis, while an inadequate request can expose patients to unnecessary interventions. This is because the test can present false-positives, due to laboratory interference or other causes of hypercalcitoninemia,[2] leading to incorrect diagnoses and conduct, with possible increases in emotional, personal and financial costs. In this sense, it is essential to emphasize that the presence of hypercalcitoninemia is not pathognomonic for MTC. Conditions that may result in hormone elevation, such as hypergastrinemia, hypercalcemia, renal failure, use of omeprazole and beta-blockers, non-thyroid neuroendocrine tumours and bacterial infections, must always be considered. In doubtful cases, particularly when calcitonin concentrations are <100 pg/mL, some authors recommend the stimulation test with pentagastrin or calcium gluconate.[2,7]

In addition to the points mentioned above, another factor to be considered when evaluating tests is their financial cost. A North American study, carried out to determine the cost-effectiveness of routine calcitonin screening in adult patients with thyroid nodules, concluded that the procedure appears to be cost-effective and comparable to measuring thyroid-stimulating hormone (TSH), colonoscopy and mammography.[12] In this regard, calcitonin tests with adequately considered

justifications, in this work, cost 2,254.24 USD. Of these, 1,973.92 USD was spent on tests requested due to the presence of a thyroid nodule. Considering the two cases where test results alerted physicians to the possibility of MTC, the cost of this supposedly earlier detection would be 986.96 USD. To get an idea of the values involved in late diagnosis, a study carried out in Germany in 2014 to evaluate the costs associated with treating advanced MTC, found that the estimated total mean cost per patient, calculated on a monthly and yearly basis were 760 euro (€) and €9,248, respectively. Considering a mean survival time of 36.7 months, lifetime costs would be €28,283.[32] Considering a euro/USD exchange rate of 1.16, the lifetime cost per patient would be 32,808.28 USD. On the other hand, in our study, the cost of calcitonin collections with inadequate or not given justifications were 417.56 USD, an amount that could have been used in screening tests for other diseases. For example, it would have been possible to carry out 95 prostate-specific antigen (PSA) tests, 69 mammograms, or 846 blood glucose tests in our laboratory at the time data was collected for this study.

This study has limitations, for instance, its retrospective nature. Thus, it was not possible to determine in all cases whether further investigation for MTC was indicated. Another limitation was the failure to perform cost-effectiveness analysis, which would have been appropriate in this type of study. Furthermore, the fact that suspicious nodules for neoplasms in general are considered an adequate justification for requesting preoperative calcitonin measurement is not a consensus. Despite these limitations, this study has merit by evaluating the reasons given for patients being referred for serum calcitonin measurements at a tertiary hospital specialized in treating thyroid cancer. Although most of the requested tests returned undetectable values, approximately 17% (n=158) of patients had detectable values. Interestingly, justifications for requesting the test were widely varied, from totally relevant, such as MTC diagnosis, to the most unexpected, such as decompensated heart failure. Thus, the findings of this study highlight the need for standardization when requesting this exam. As this method is available and accessible to students, residents and physicians from all specialties in the service in question, any of them could request the test according to their own criteria. And, worryingly, a higher percentage of cases with inadequate or no justification given for the test presented detectable calcitonin values than those with adequate justification for the test. Some of these cases even showed high calcitonin concentrations, which could raise suspicion of MTC being present.

CONCLUSION

This study demonstrated that a considerable proportion of serum calcitonin measurement requests have been inadequately justified or lacked justification. A higher prevalence of detectable hormone levels was observed in these cases and among patients with MTC. Thus, health services must establish specific protocols for requesting the test to avoid the negative consequences of unnecessary investigations and false positive results.

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REFERENCES

1. Schlumberger MB, Dralle H, Jarzab B, et al. 2012 European Thyroid Association guidelines for metastatic medullary thyroid cancer. Eur Thyroid J. 2012;1(1):5-14. doi:10.1159/000336977
2. Maia AL, Siqueira DR, Kulcsar MA, et al. Diagnosis, treatment, and follow-up of medullary thyroid carcinoma: recommendations by the Thyroid Department of the Brazilian Society of Endocrinology and Metabolism. Arq Bras Endocrinol Metabol. 2014;58(7):667-700. doi:10.1590/0004-2730000003427
3. Wells SA Jr, Asa SL, Dralle H, et al. Revised American Thyroid Association guidelines for the management of medullary thyroid carcinoma. Thyroid. 2015;25(6):567-610. doi:10.1089/thy.2014.0335
4. Essig GF Jr, Porter K, Schneider D, et al. Fine needle aspiration and medullary thyroid carcinoma: the risk of inadequate preoperative evaluation and initial surgery when relying upon FNAB cytology alone. Endocr Pract. 2013;19(6):920-7. doi:10.4158/EP13143.OR
5. Vitale G, Caraglia M, Ciccarelli A, et al. Current approaches and perspectives in the therapy of medullary thyroid carcinoma. Cancer. 2001;91(9):1797-808. doi:10.1002/1097-0142(20010501)91:9<1797::aid-cncr1199>3.0.co;2-p
6. 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):394-8. doi:10.1002/dc.21731
7. Toledo SPA, Lourenco DM Jr, Santos MA, et al. Hypercalcitoninemia is not pathognomonic of medullary thyroid carcinoma. Clinics (Sao Paulo). 2009;64(7):699-706. doi:10.1590/S1807-59322009000700015
8. Unluhizarci K, Akgun H, Oz B, et al. Patients with papillary thyroid carcinoma associated with high stimulated serum calcitonin levels. Endocrinol Diabetes Metab Case Rep. 2017;2017:17-0085. doi:10.1530/EDM-17-0085
9. Uccella S, Blank A, Maragliano R, et al. Calcitonin-producing neuroendocrine neoplasms of the pancreas: clinicopathological study of 25 cases and review of the literature. Endocr Pathol. 2017;28(4):351-61. doi:10.1007/s12022-017-9505-4
10. Lupoli GA, Barba L, Liotti A, et al. Falsely elevated thyroglobulin and calcitonin due to rheumatoid factor in non-relapsing thyroid carcinoma: a case report. Medicine (Baltimore). 2019;98(5):e14178. doi:10.1097/MD.0000000000014178

11. Hodak SP, Burman KD. The calcitonin conundrum: is it time for routine measurement of serum calcitonin in patients with thyroid nodules? J Clin Endocrinol Metab. 2004;89(2):511-4. doi:10.1210/jc.2003-031983
12. Cheung K, Roman SA, Wang TS, et al. Calcitonin measurement in the evaluation of thyroid nodules in the United States: a cost-effectiveness and decision analysis. J Clin Endocrinol Metab. 2008;93(6):2173-80. doi:10.1210/jc.2007-2496
13. 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):163-8. doi:10.1210/jc.2003-030550
14. Torresan F, Mian C, Cavedon E, et al. Cure and survival of sporadic medullary thyroid carcinoma following systematic preoperative calcitonin screening. Langenbecks Arch Surg. 2019;404(4):411-9. doi:10.1007/s00423-019-01764-3
15. Machens A, Schneyer U, Holzhausen HJ, et al. Prospects of remission in medullary thyroid carcinoma according to basal calcitonin level. J Clin Endocrinol Metab. 2005;90(4):2029-34. doi:10.1210/jc.2004-1836
16. Instituto Brasileiro de Geografia e Estatística (IBGE). Censo Demográfico 2010: características gerais da população, religião e pessoas com deficiência. Rio de Janeiro: IBGE; 2012. Available from: https://biblioteca.ibge.gov.br/visualizacao/periodicos/94/cd_2010_religiao_deficiencia.pdf.
17. Lloyd RV, Osamura RY, Klöppel G, et al, editors. WHO classification of tumours of endocrine organs. 4th ed. Lyon, France: IARC Press; 2017.
18. Hauache OM, Vieira JG, Maciel RMB. Diagnóstico laboratorial do carcinoma medular de tireoide: calcitonina basal e testes de estímulo. Arq Bras Endocrinol Metabol. 2003;47(5):529-33. doi:10.1590/S0004-27302003000500005
19. Wang TS, Roman SA, Sosa JA. Detection of medullary thyroid cancer: a focus on serum calcitonin levels. Expert Rev Endocrinol Metab. 2008;3(4):493-501. doi:10.1586/17446651.3.4.493
20. Soh SB, Aw TC. Laboratory Testing in Thyroid Conditions - Pitfalls and Clinical Utility. Ann Lab Med. 2019;39(1):3-14. doi: 10.3343/alm.2019.39.1.3
21. Kwon H, Kim WG, Choi YM, et al. A cut-off value of basal serum calcitonin for detecting macroscopic medullary thyroid carcinoma. Clin Endocrinol (Oxf). 2015;82(4):598-603. doi:10.1111/cen.12562
22. Allelein S, Ehlers M, Morneau C, et al. Measurement of basal serum calcitonin for the diagnosis of medullary thyroid cancer. Horm Metab Res. 2018;50(1):23-8. doi:10.1055/s-0043-122237
23. Frank-Raue K, Schott M, Raue F. Recommendation for calcitonin screening in nodular goiter. Dtsch Med Wochenschr. 2018;143(15):1065-9. doi:10.1055/a-0585-8097
24. Filimon S, Payne RJ, Black MJ, et al. Calcitonin secretory index and unsuspected nodal disease in medullary thyroid carcinoma. Endocr Pract. 2018;24(5):460-7. doi:10.4158/EP-2017-0236
25. Cohen R, Campos JM, Salaün C, et al. Preoperative calcitonin levels are predictive of tumor size and postoperative calcitonin normalization in medullary thyroid carcinoma. J Clin Endocrinol Metab. 2000;85(2):919-22. doi:10.1210/jcem.85.2.6556
26. Sand M, Gelos M, Sand D, et al. Serum calcitonin negative medullary thyroid carcinoma. World J Surg Oncol. 2006;4:97. doi:10.1186/1477-7819-4-97
27. Trimboli P, Giovanella L. Serum calcitonin negative medullary thyroid carcinoma: a systematic review of the literature. Clin Chem Lab Med. 2015;53(10):1507-14. doi:10.1515/cclm-2015-0058
28. Woliński K, Kaznowski J, Klimowicz A, et al. Diagnostic value of selected biochemical markers in the detection of recurrence of medullary thyroid cancer: comparison of calcitonin, procalcitonin, chromogranin A, and carcinoembryonic antigen. Endokrynol Pol. 2017;68(4):434-7. doi:10.5603/EP.a2017.0038
29. Trimboli P, Mian C, Piccardo A, et al. Diagnostic tests for medullary thyroid carcinoma: an umbrella review. Endocrine. 2023;81(2):183-93. doi: 10.1007/s12020-023-03326-6
30. Gambardella C, Offi C, Patrone R, et al. Calcitonin negative medullary thyroid carcinoma: a challenging diagnosis or a medical dilemma? BMC Endocr Disord. 2019;19(Suppl 1):45. doi:10.1186/s12902-019-0367-2
31. Rosario PW, Penna GC, Brandão K, et al. Usefulness of preoperative serum calcitonin in patients with nodular thyroid disease without suspicious history or cytology for medullary thyroid carcinoma. Arq Bras Endocrinol Metabol. 2013;57(4):312-6. doi:10.1590/s0004-27302013000400006
32. Kreissl MC, Jacob C, Führer D, et al. Best supportive care from the conservative/non-surgical perspective and its costs in the treatment of patients with advanced medullary thyroid cancer: results of a Delphi panel. Oncol Res Treat. 2014;37(6):316-22. doi:10.1159/000362613