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RESEARCH ARTICLE

Evaluation of calcitonin measurement in fine needle aspirates in the diagnosis of primary and metastatic medullary thyroid carcinoma

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SUMMARY

Serum CT represents the tumoral marker of medullary thyroid carcinoma (MTC), and is a main diagnostic tool and follow-up after treatment. Nevertheless, calcitonin levels can be elevated in a number of other situations and does not always distinguish MTC from other clinical circumstances, particularly when elevation remains moderate. Fine needle aspiration biopsy is a widely used examination for the investigation of thyroid nodules and lymph nodes, but its sensitivity for the diagnosis of MTC remains low ranging from 45% to 63%. Calcitonin measurement in fine needle aspiration wash out fluid has been reported as reliable, and is recommended by the experts of American Thyroid Association (ATA) in their 2015 consensus for the management of MTC even if its diagnostic is not standardized yet.

Material and methods: Twenty patients with 45 lesions (thyroid nodule and lymph node) were included in this study; calcitonin was performed in each of them, the puncture needles were washed with 1ml of saline solution to obtain to measure Calcitonin in fine needle aspiration washout fluid.

Results: After surgery 32 MTC were diagnosed on histology. Receiver operating curve (ROC curve) indicated CT in fine needle aspiration >22.99pg/ml as more accurate cut off value with a sensitivity and specificity of 96.87% and 100%. Calcitonin ratio was evaluated too, the cut off value being>0.64 with a sensitivity of 62.5% and specificity of 100%.

Conclusion: Calcitonin in fine needle aspiration wash out fluid constitutes an excellent diagnostic tool of MTC with the use of 22.99pg/ml threshold.

Keywords: Medullary thyroid carcinoma, calcitonin, calcitonin in fine needle aspiration biopsy.

Introduction

Medullary thyroid cancer (MTC) is a rare, slowly progressing neuroendocrine tumor, that originates from para follicular C cells secreting calcitonin (CT). 75% of MTC is a sporadic tumor when 25% is a familial disease integrated within multiple endocrine neoplasia 2. This cancer represented 5 to 10% of thyroid neoplasm according the series. Its incidence in nodular thyroid pathology is around 0.3 and 2.85% depending on the series[1,2,3,4,5,6,7,8,9]. The prognosis depends mainly on the tumor stage at diagnosis and initial surgical treatment. Serum CT (sCT) represents the tumoral marker of MTC; and is a main diagnostic tool and follow-up after surgery. Nevertheless, it can be elevated in a number of other situations including certain medications, alcohol consumption, smoking, pregnancy hypergastrinemia and in certain pathologies such as neuroendocrine tumors, differentiated thyroid cancers and renal insufficiency. As a result, sCt does not always distinguish MTC from other conditions, especially when it is slightly elevated. Patient With confirmed MTC should have a total thyroidectomy depending on the preoperative sCT and lateral the extent of the disease, additional dissection of the lymph node in the central and lateral neck compartment. Fine needle aspiration biopsy (FNAB), a widely used examination for thyroid nodules and lymph nodes, has a suboptimal sensitivity for the diagnosis of MTC, ranging from 45%to 63 %[10,11,12,13]. The calcitonin measurement in fine needle aspiration biopsy wash-out fluid (CT-FNA) has been report in many studies as highly reliable and has been largely and rapidly diffuse, Although, the CT-FNA has not yet been standardized. This study was aimed to develop a decisional cut-off of CT-FNA and CT-FNA/sCT ratio for the diagnosis of MTC.

Material and methods

We conducted a prospective study; patients were recruited over 3 years (2017-2019). We included 2 groups of patients. Group 1 comprised patients with nodular or multi-nodular goiter in need of surgery, with sCT level>10pg/ml (threshold of the dosing kit). They were included after ruling out functional or secondary hypercalcitoninemia due to anotherpathology (neuroendocrine tumor of the lung or gastrointestinal tract, Hashimoto disease, sepsis, chronic renal failure, hypergastrinemia, pseudohypoparathyroidism). Group 2 patients were those being followed up for previously MTC with a persistence of elevated sCT> 10pg/ml.

The patients refusing to participate to the study, refusing FNAB, or having extra nodal metastases were excluded.

Ultrasonography

Ultrasonography (US) of the neck or cervical ultrasonography was performed on all patients using Toshiba Aplio 400 (Toshiba medical systems corporation, Japan), with exploration of the thyroid (group 1) and cervical lymph nodes (group 1 and 2) using a high frequency probe (10-12 MHz) linear electronic transducer. Thyroid nodule malignancy criteria were specified.

Fine needle aspiration biopsy and calcitonin in n=fine needle aspiration wash out fluid

The fine needle aspiration biopsy was carried out directly on a nodule/lymph node or guidance by US, with a 25gauge needle attached to 10 ml syringe. The needle is moved back and forth

several times, so that the material penetrates the needle lumen by capillary action, with one or two passes per lesion. The sample is spread on 3 or 4 slides, air-dried and stained with May-Grunwald- Giemsa (MGG) before being read under the light microscope. The interpretation of the result was made by only one experienced thyroid cytopathologist who studied all specimen. The results of sCT were not communicated to him for blind and independent analysis.

After smear preparation, the needle was washed out with 1 ml of normal saline solution, the resulting product is stored at -20°C until the time of dosing.

Laboratory assays

Serum TSHus and anti TPO antibody (Ab-TPO) were measured by automate ARCHITECT I 1000SR ABBOTT System. The sCT and CT-FNAB were determined by immune radiometric assay (CISBIO International Bio-assays IRMA hCT) with functional sensitivity of 4 pg/ml; analytical sensitivity of 1.5pg/ml and normal range 0-10pg/ml.

Surgery

In the group 1, a total thyroidectomy ±lymph node dissection was performed according to the presence of cervical lymph nodes. In the second group, the patients were included for lymph nodes dissection if the sCt remains high after surgery with cervical lymph node in ultrasound or if first surgery was incomplete or inadequate.

Histology and immunohistochemistry/ immunostaining

Following thyroidectomy and or lymph node dissection; the histological examination coupled with the immunohistochemistry on surgical specimen was carried, and is therefore the gold standard for this study. To prove MTC, we used an anti- CT polyclonal antibody and an anti-chromogranin polyclonal antibody from DAKO.

Ethics

All patients were informed about the protocol of this study; then signed a letter of an informed consent before inclusion. This work has been approved by the scientific committee of Tlemcen faculty of medicine (Algeria).

Definitions

True positive (TP) was defined as the correct prediction of primary and/or recurrent MTC; true negative (TN) as the correct prediction of no MTC; false positive (FP) as the incorrect prediction of disease with histological examination negative from MTC; and false negative (FN) as the incorrect prediction of no MTC with histological evidence of MTC.

Statistical analysis

Data were collected and analysed using 'Statistical social Package for the sciences' (SPSS 21 for Windows SPSS Inc, Chicago, IT, the USA) and Statistics for Biomedical Research software (MedCal 18 for Mariakerke; Belgium). We determined frequencies for qualitative variables and means ± standard deviation, minimum and maximum for quantitative variables.

The normality of sCT and CT-FNA distribution was assessed using the Shapiro-Wilk test, since the value was normally; the Student and Anova test was applicated and when the value was not normally; the non parametrics tests such as: The Mann-Whitney and Kruskall-Wallis

test were performed. For all comparisons; *p* values<0.05 were considered statistically significant. Spearman rank correlation test was applicated to verify the relation between two quantitative variables. It is considered significant if *p*< 0.05.

The Receiver operating characteristic (ROC) curve was used to appreciate the diagnostic performance such as Sensitivity (Se) and Specificity (Sp) of the CT- FNA and the CT ratio. The ROC curve was performed to determine the cut-off of CT-FNA and CT ratio. This ratio was evaluated to avoid the risk of over estimation of CT-FNA following contamination by the sCT during the puncture.

The positive predictive value (PPV) and negative predictive value (NPV) for each test are calculated using the following formulas: PPV= TP/TP+FN; NPV=TN/TN+FP.

The Youden index [(Se-Sp)-1] is calculated for the CT-FNA and CT ratio. This index is considered efficient if it is closer to 1. The Yule Q coefficient [Q= (TP x TN- FN x FP) / (TP x TN+ FN x FP) is also calculated for the 2 tests and assessed the link between 2 variables (test/disease); if the value tends to 1, the relationship between the parameters is stronger.

A comparative study of the diagnostic performance of CT-FNA and CT ratio will be carried out by comparing the 2 ROC curves or more precisely the 2 AUC according to Hanley and McNeil method. Significance was set at *p*<0.05. The index Kappa (K) evaluating the agreement between 2 tests, is evaluated for several pairs of tests. The closer the value of this index is to 1, the greater the concordance of results.

Results

A series of 20 patients were included in the study (5 women and 15 men) with a mean age of 51.75 ±14.45 (28 to77years). The mean value of sCT in all patients was 1058.30 ± 1048.0 pg/ml (16.88 to 4080). The mean sCT in group1 and 2 was respectively 2005.43±2000.70 pg/ml (25.78-4080) and 821.5±540.17 pg/ml (16.88-3332.94) without significant difference (*p*=0.32).

Ultrasonography was performed in all patients; it found 119 lesions (109 neck lymph nodes, 8 thyroid nodules and 2 suspicious neck recurrences). The mean lesion size was 21.94 ± 11.65mm (5 to 60mm). The mean measurements of thyroid nodule; lymph nodes and neck mass were 25.96±15.83mm, 20.62± 10,74mm and 29.00±1,41mm with no significant difference (*p*=0.35).

Cytological examination was performed into only 45 lesions (35 lymph nodes, 8 thyroid nodules and 2 suspicious neck recurrences); the others were not accessible because of their deep localization or near to a blood vessel. The CT-FNA was measured in all lesions.

After surgery, histological examination ± immunohistochemistry confirmed MTC in 32/45 lesions (one mixed carcinoma: MTC and papillary thyroid carcinoma). For the remaining lesions one showed follicular thyroid carcinoma and 12 lesions were benign (Table1). No C Cell hyperplasia was found.

Table 1 : The result of cytology, CT-FNA and CT ratio in different lesions

Test	Histology (CMT +)	Histology (CMT-)
Cytology		
FNAB+	17	1
FNAB -	15	12
CT in-situ		
CT-FNA +	31	1
CT-FNA -	0	13
Ratio CT		
Ratio +	20	0
Ratio -	12	13

The cytological examination was in favor of malignancy in 32/45 lesions (71%) but recognized MTC in only 18/45lesions (40%). Cytology correctly diagnosed MTC in 17/45 lesions represented TP, one lesion represented FN.TN results were observed in 12 lesions and 15 lesions were FN (Table 1). For cytology; Se was 53.12%, Sp92.30%; PPV 94.44% and NPV 44.44% (Table 2).

Table 2: The performances diagnostic of different tests

Test	value	Ss (%)	Sp (%)	PPV (%)	NPV (%)	Youden index	Coefficient (Q) of Yule
CT- FNA	>22,99pg/ml	96,87	100	100	92,9	0,9688	1
Ratio CT	>0,64	62,5	100	100	52	0,625	1
Cytology (FNAB)	-	53,12	92,3	94,44	44,44	-	-

The CT-FNA was >10pg/ml in 78% of the lesion, the mean value was 903.8±1572.64 pg/ml (0.21- 9690.13). It was 1268.09 ±1742.44 pg/ml (18,5- 9690.13) in the MTC lesions and 7.01±6.09 pg/ml (1.13- 22.99) in the benign lesions with a significant difference (p=.000).

ROC curve for CT-FNA showed an area under the curve (AUC) =0.998(p<0.0001) (figure1). The inflection point of the curve corresponds to the cut-off =22.99pg/ml. The value offering the best compromise between Se (96.87%) and Sp (100%). The Youden index = 0.96 (near to 1), the Yule Q coefficient = 1 indicating a close relationship between the positivity of the test and the existence of the disease. With the cut-off of 22.99pg/ml; the CT-FNA correctly diagnosed MTC in 31 lesions; there was one FN, no FP and 13TN (table 1). PPVwas100% and NPV was 92.9% (table 2).

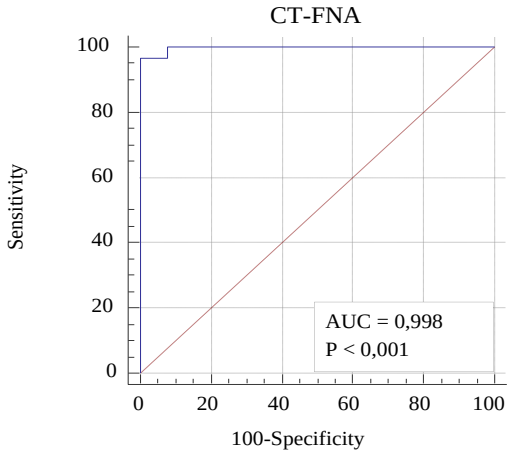

Figure 1: ROC curve for CT-FNA

The mean value of CT ratio was 0.96± 1.58 (0 - 9.7) with a significant difference (p=, 000) between the mean in the benign lesions in histology (0.16±0.21) and MTC lesion (1.28±1.78).

The ROC curve established for the CT ratio showed an AUC= 0.889 (p< .0001) (figure 2). The more accurate cut-off of CT ratio was 0.64; leading to Se of 62.5% and Sp of 100%. According to this cut-off CT ratio correctly diagnosed 20 lesions; 12 represent FN and it did not have FP (Table1). The PPV is 100%, however, NPV is only 52% (table2). Youden index =0.625 indicates that the CT ratio remains an interesting parameter for the diagnosis of MTC. The Yule coefficient (Q) =1 confirm the results obtained.

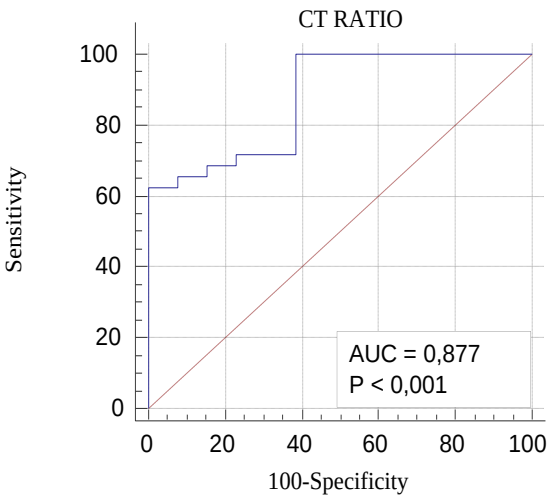

Figure 2: ROC curve for CT Ratio

The comparison of the two ROC curves (CT-FNA/sCT ratio) with Hanley's and Mc- Neil's test finds a significant difference between the 2 parameters (p=0.02) and concluded that CT-FNA is more performing than the CT ratio for MTC diagnosis. Thekappa index of the CT-FNA / Histology pair was with a value of 0.94 proving the excellent agreement between these two exams. This index was 0.49 between CT ratio/histology and 0.34 between cytology and histology.

Finally, we found a significant correlation between the CT-FNA and sCT (r= 0.53, p=.000), between CT-FNA and ACE (r= 0.32, p=0.02) and between CT-FNA and the CT ratio (r=0.83, p=.000).

Discussion

The challenge for the clinician is to make a definite preoperative diagnosis of MTC, since this is the key to surgical act and consequently, to the prognosis of the disease. The sCT and cytology alone are not sufficient to diagnose MTC in all cases. CT- FNAB is another diagnostic tool for MTC used by several endocrinologists but its cut-off remains a matter of debate.

Several studies, with different methodologies have set out to assess the Se and the Sp of the CT-FNAB and have tried to establish a cut-off for this test. The first was the study of Boi et al [14] who retrospectively examined 36 lesions (18 Thyroid nodules and 18 lymph nodes) in 23 patients with increased sCT. MTC has been confirmed in 21/36 lesions. The authors propose an arbitrary cut- off of 36 pg/ml corresponding to three times the highest CT-FNAB in benign lesions. The Se and Sp are estimated at 100%. In this study a good correlation has been found between sCT and CT-FNAB (r=0.64, p<0.009).

Diazzi et al.[15] has published in 2014 a prospective study of 27 patients presenting 60 thyroid nodules, only 18/27 patients had a real suspicion of MTC with a high sCT. According to this study, if CT-FNAB is less than 17 pg/ml, MTC can be ruled out and if CT-FNAB is over than 1000 pg/ml, MTC

becomes certain; however, things are unclear for values ranging between 17 and 1000 pg/ml. Indeed, these rates can correspond with differentiated thyroid carcinoma, HCC or real MTC. The results thus exposed do not help really the clinician in decision-making process and does not bring any further arguments for the diagnostic approach. The study of Trimboli et al[10] included 36 patients (34 nodules and 4 lymph nodes) and 52 non-medullary lesions from subjects undergone biopsy (control group), it proposes a cut-off of CT-FNAB of 39,6 pg/ml corresponding to high sCT plus inter-laboratory CV (15,6) with this threshold CT-FNAB had a sensitivity and a specificity of100%.

In addition, Kudo et al.[16] confirms the MTC with this technique in 5 patients and proposes an arbitrary cut-off of 67 pg/ml (the highest value of control group). Massaro et al.[17] evaluating this technique among 27patients with sCT relatively high and 37 normal sCT, found no significant difference in CT-FNAB between the two groups and no correlation between sCT and CT-FNAB and concluded that this method does not have the same diagnostic performance as thyroglobulin in the fine needle aspiration wash-out in the diagnosis of metastases of differentiated thyroid cancers.

The study of De-Crea et al[18,] was a prospective one; whose methodology inspired our work, included 38 patients with 62 lesions, 18 corresponded to MTC after histology. The ROC curve established to evaluate the diagnostic performance of the CT-FNAB has found a threshold of 10.4 pg/ml with a Se of 89% and Sp of 100%. This study is the only one which was interested in the CT ratio whose cut-off estimated at 1.39 with a Se of 83%, Sp of 93%.

In Zamira study[19], CT-FNAB offered a Se of 92.5% and Sp of 100% with a cut-off > 122 pg/ml for thyroid nodule and > 35.8 pg/ml for lymph node. These results were confirmed in a recent Turkish study[20] with a different threshold.

In our series, and in order to appreciate the place of the CT-FNA in the diagnostic Approach of the MTC, we included only patients with high sCT and a well-founded suspicion of MTC or recurrence of MTC. The analysis found a significant correlation between the CT-FNA and sCT (r =0.53, *p*=.000), between CT-FNA and ACE (r =0.32, *p*=0.02) and between CT-FNA and CT ration (r=0.83, *p*=.000) as in the study of Trimboli [10] and Boi[14].

The ROC curve indicated that the CT-FNA is an excellent tool of diagnostic with a surface AUC = 0.998, the sensitivity of 96.87% and the specificity of 100% for a cut-off of 22.99 pg/ml. The Youden index =0.96, indicates that the CT-FNA is an exam with excellent diagnostic value, the coefficient of Yule =1 indicates that there is a good relationship between the positivity of the CT-FNA and the existence of MTC. For the CT ratio, the Ss and SP are respectively of 100% and 52%, its diagnostic performance remains acceptable but less interesting than the CT-FNA as indicated by Hanley test.

In our study, the cytology has Se =53.12% and Sp =92.30% according to numerous studies and in the meta-analysis of Trimboli et al.[21] which estimated Ss of the cytology at 56%. The same results are reported in a recent meta-analysis[22]. Most of studies have concluded that le Se of CT-FNA was significantly superior to that of FNA-cytology in the diagnosis of MTC[22, 23, 24].

Conclusion

The CT-FNA constitutes an excellent diagnostic tool of MTC, and its must be performed alongside the cytology. Comparison of the results of these two exams with those of sCT increases the chances of diagnosing MTC before surgery, in particular when cytology is non- contributory and sCT is not clearly elevated.

Statement of Ethics:

Our study is in accordance with the ethical standard so four institutions and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. All the patients were informed about the protocol of this study; they signed an informed consent letter before inclusion.

Conflict of Interest Statement:

The authors declare no conflict of interest.

Competing Interests:

The authors declare no competing interests.

Funding Statement:

None

Author Contributions:

All authors contributed to the design, management of the study, and written manuscript.

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