Thyroid

Calcitonin measurement in fine-needle aspirate washouts *vs*. cytologic examination for diagnosis of primary or metastatic medullary thyroid carcinoma

Dosaggio della calcitonina nel liquido di lavaggio dell'agoaspirato vs. esame citologico nella diagnosi del carcinoma midollare della tiroide primitivo o metastatico

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SUMMARY

Ultrasound-guided fine-needle aspiration biopsy cytology (FNAB-C) is able to detect approximately 63% of medullary thyroid carcinoma (MTC). The measurement of calcitonin in the needle washout (FNAB-CT) could improve its accuracy. Sixty-two FNAB-C were performed in 38 patients. Serum calcitonin (sCT) was measured before performing FNAB-C. After obtaining a FNAB-C specimen, the needle was washed with 0.5 ml of saline solution to obtain the CT washouts. Receiver operating characteristic (ROC) analysis identified the cut-offs of FNAB-CT and FNAB-CT/sCT. Eighteen MTC were found at final histology. ROC analysis indicated FNAB-CT > 10.4 pg/ml and FNAB-CT/sCT > 1.39 as more accurate cut-off values. Overall accuracy, positive (PPV) and negative predictive values (NPV) were 85%, 100 and 83%, respectively, for FNAB-C, 97%, 100%, 96% for FNAB-CT and 90%, 83% and 93% for FNAB-CT/sCT. The integration of FNAB-C and FNAB-CT resulted in 98% overall accuracy, 100% PPV and 98% NPV; the integration of FNAB-C and FNAB-CT/sCT in 90% overall accuracy, 80% PPV and 95% NPV. One of 2 false negative FNAB-CT and one of 3 false negative FNAB CT/sCT were correctly diagnosed by FNAB-C. Eight of 9 non-diagnostic FNAB-C were correctly classified by FNAB-CT and 7 by FNAB CT/sCT. FNAB-CT should integrate but not replace FNAB-C. FNAB-CT is particularly useful in the presence of non-diagnostic FNAB-C.

KEY WORDS: Medullary thyroid carcinoma • Calcitonin • Lymph node metastases • US-guided fine-needle aspiration biopsy • Cytology

RIASSUNTO

L'esame citologico su agoaspirato ecoguidato con ago sottile (FNAB-C) rappresenta una delle procedure più comuni per la conferma della diagnosi di carcinoma midollare della tiroide (CMT) primitivo e/o metastatico. Tuttavia la sensibilità riportata per questa metodica nella diagnosi del CMT è di circa il 63%. Il dosaggio della calcitonina nel liquido di lavaggio dell'agoaspirato (FNAB-CT) è una metodica recentemente introdotta, proposta al fine di migliorare l'accuratezza diagnostica della citologia convenzionale. Sono stati considerati tutti i pazienti con sospetto CMT primitivo e/o metastatico sottoposti a FNAB-C e FNAB-CT tra Marzo 2012 e Settembre 2013, per i quali era disponibile la conferma istologica. La calcitonina sierica (sCT) è stata dosata prima dell'esecuzione del FNAB-C. Dopo aver prelevato e preparato il campione per l'esame citologico, l'ago è stato lavato con 0,5 ml di soluzione salina per ottenere il dosaggio della CT nel liquido di lavaggio. È stata eseguita un'analisi ROC al fine di identificare i cut-off con più elevata sensibilità ed accuratezza rispettivamente per il FNAB-CT e il rapporto tra FNAB-CT e sCT (FNAB-CT/sCT ratio). L'accuratezza diagnostica dei cut-off stabiliti è stata confrontata con quella del FNAB-C. Sono stati eseguiti 62 FNAB-C in 38 pazienti. L'esame istologico definitivo ha confermato la diagnosi di CMT in 18 lesioni (29,9%). L'analisi ROC ha individuato un valore > 10,4 pg/ml e > 1,39 come cut-off più accurati rispettivamente per FNAB-CT e FNAB-CT/sCT ratio. L'accuratezza, il valore predittivo positivo (VPP) ed il valore predittivo negativo (VPN) sono risultati, rispettivamente, 85%, 100% e 83% per il FNAB-C, 97%, 100% e 96% per il FNAB-CT e 90%, 83% e 93% per FNAB-CT/sCT ratio. L'integrazione di FNAB-C e FNAB-CT ha mostrato una accuratezza pari al 98%, un VPP pari al 100% ed un VPN pari al 98%; l'integrazione di FNAB-C e FNAB-CT/sCT ratio ha mostrato un'accuratezza del 90%, un VPP dell'80% ed un VPN del 95%. Il FNAB-C ha identificato correttamente 1 dei 2 casi risultati falsi negativi al FNAB-CT e 1 dei 3 casi falsi negativi al FNAB-CT/sCT ratio. La procedura del FNAB-CT ha diagnosticato correttamente 8 dei 9 casi non diagnostici al FNAB-C, mentre il FNAB-CT/sCT ratio ne ha individuati correttamente 7. Nella nostra esperienza il FNAB-CT è risultato più accurato del FNAB-CT/sCT ratio. Nella diagnosi del CMT primitivo o metastatico il FNAB-CT può integrare ma non sostituire il FNAB-C ed è particolarmente utile nei casi non diagnostici alla citologia convenzionale.

PAROLE CHIAVE: *Carcinoma midollare della tiroide • Calcitonina • Metastasi linfonodali • Agoaspirato ecoguidato • Esame citologico*

Introduction

Serum calcitonin (sCT) is a key marker in diagnosing medullary thyroid carcinoma (MTC) [1-5] and has been demonstrated to be highly sensitive for differential diagnosis, prognostic assessment, follow-up, and evaluation of treatment response in MTC [3 5 6]. Routine measurement of sCT has been investigated as a screening method for diagnosis of MTC in patients with thyroid nodules [6 7] with the reported advantages of early diagnosis [6 8 9] and improved 10 year outcome of MTC patients diagnosed with sCT screening [8 9]. However, false-positive rates for basal sCT testing remain high and positive predictive value (PPV) low, even with the most recent ultrasensitive assays [6]. As a consequence, the routine sCT measurement in patients with thyroid nodules is still debated and the American Thyroid Association (ATA) has declined to make any recommendation for or against sCT screening [2].

Recently, several European consensus groups recommended sCt measurement routinely [5] or suggested its use in specific conditions (e.g. subjects with family history of MTC, cytology suggestive of MTC or undergoing surgery for goitre) [4].

Since sCT is not helpful in localising primary tumours in the thyroid gland and/or its neck recurrences/metastases in thyroidectomised patients, the localisation of disease should start with careful neck ultrasound examination [2 3 10]. Although MTC can be diagnosed by ultrasound-guided fine-needle aspiration biopsy cytology (FNAB-C) based on typical pathological features [7], the sensitivity of FNAB-C has been demonstrated to be 45-63%, indicating that misdiagnosis often occurs with this approach [11]. Recently, several published studies have demonstrated that high CT concentrations were present in the wash-out of the needle used for FNAB-C both in suspicious lymph nodes and in thyroid nodules histologically confirmed to be metastases or primary MTCs, respectively [2 7 12-15].

Further studies [16-18] showed that the measurement of CT in the needle washout (FNAB-CT) had high sensitivity and specificity in diagnosis of MTC. A major concern in this approach is the cut-off to be used to define the positive value of CT in the washout fluid. This problem is of course strictly dependent on the sCT level that can contaminate the needle and lead to misinterpretation of results. Moreover, there is still no univocal interpretation of the role of FNAB-CT and its correlation with sCT or with FNAB-C. We aimed to prospectively evaluate the accuracy of FNAB-CT or FNAB-CT/sCT ratio, alone or integrated with the results of FNAB-C, in diagnosis of primary or metastatic medullary thyroid carcinoma.

Materials and methods

All patients with suspicious primary and/or recurrent/metastatic MTC who underwent FNAB-C and FNAB-CT before initial surgery or during post-surgery follow-up between March 2012 and September 2013 were considered. Only patients in whom histological evaluation was obtained were included in this study.

All patients underwent to sCT measurement before FNAB-C. After obtaining the cytological specimen by ultrasound (US) guided FNAB, the CT washout was performed.

Demographic, laboratory, clinical, operative, pathologic and follow-up data were prospectively registered for all patients.

Study endpoints

The primary endpoint of the study was to evaluate the accuracy of FNAB-CT and FNAB-CT/sCT ratio in diagnosis of MTC. The secondary endpoint of the study was to evaluate the validity of the integration of FNAB-C with FNAB-CT and FNAB-CT/sCT ratio.

US and US-FNAB

Ultrasonography was performed using a Toshiba Aplio 400 ultrasound instrument (©Toshiba Medical Systems Corporation, Tochigi-ken, JAPAN). A complete neck ultrasonographic mapping, including the thyroid, central and lateral neck node compartments and level of the thyroidectomy scar in thyroidectomised patients, with a high-frequency (10-12 MHz) probe was performed in all patients. According to the available guidelines [1-5], the ultrasound thyroid/neck mass features considered suspicious were: solid aspect, hypoechogenicity, microcalcifications, irregular margins or absent halo sign, intranodular vascularisation and shape (taller than wide) [1-5 19]. The ultrasound lymph nodes features considered suspicious were: loss of echogenic hilum, hyperechogenicity, cystic changes, calcification, abnormal vascularity, heterogeneous echogenicity and a round shape (longitudinal/transverse diameter ratio < 1.5) [1-5 20].

All FNAB were performed under US guidance, using a 21-23 gauge needle. Each lesion was aspirated at least twice. Immediately after the first aspiration, after obtaining a FNAB-C specimen, the needle was washed with 0.5 ml of saline solution and the washout was submitted for CT measurement. All US examinations and US-FNAB were performed by an experienced endocrine surgeon or by a resident under supervision.

Receiver operating characteristic (ROC) analysis was performed to determine the absolute cut-off levels of CT in the washing fluid and the cut-off ratio between FNAB-CT and sCT with the highest sensitivity and accuracy. Diagnostic accuracies of the established cut-offs were compared with that of FNAB-C.

Cytological analysis

The cytologic specimen was prepared using a liquid-

based cytology technique based on a two-step procedure: I) fixation of the material in a methanol-based solution and II) automated processing of the material to obtain a thin layer of cells with a computer-assisted device. The aspirated material is fixed with the haemolytic and preservative methanol-based solution Cytolyt™ (Cytyc Co.) after rinsing the needle in this solution. The cells were spun and the sediment was transferred in the Preservcyt™ (Cytyc Co.) solution to be processed with the ThinPrep 2000™ automated processor. The resulting slide was fixed in 95% ethanol and stained with Papanicolaou, while the remaining material was stored in the Preservcyt™ solution to be used for eventual additional investigations [21]. For each case a thin-layer cytology slide and a series of conventional smears were made either with two different needle passes or with the split-sample technique. All conventional smears are fixed in 95% ethanol and stained with Papanicolaou [22].

The interpretation of FNAB-C was performed by dedicated cytopathologists in thyroid cytology. For the purpose of the study, the results of cytology were classified in two diagnostic categories: negative in case of inadequate/non-diagnostic samples and benign cytology; positive in case of samples with typical features for MTC or MTC metastases.

CT testing

CT was measured with a chemiluminescence immunoassay (CLIA) using a Liaison XL instrument (DiaSorin) with a functional sensitivity of 3 pg/ml. Functional sensitivity is the concentration measurable with a coefficient of variation, CV < 20% interpoled on the imprecision profile built from different sera, at different levels of CT concentration, assayed periodically in a defined period of time. The use of highly sensitive assay has enabled the low cut-off selection.

Definitions

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

Statistical analysis

Statistical analysis was performed using a commercially available statistic software package (SPSS 15.0 for Windows® - SPSS Inc., Chicago, IL, USA). The chi-squared test was used for categorical variables, while a Student's t-test was used for continuous variables. A p value < 0.05 was considered significant.

The diagnostic performance, including sensitivity, specificity, accuracy, PPV, and negative predictive value (NPV), was evaluated. Sensitivity [TP/(TP+FN)], specificity [TN/(TN+FP)], positive predictive value (PPV) [TP/(TP+FP)], negative predictive value (NPV) [TN/(TN+FN)], and overall accuracy [(TP+TN)/(TP+TN+FP+FN)] of FNAB-C, FNAB-CT, FNAB-CT/sCT ratio and of the integration of FNAB-C respectively with FNAB-CT and FNAB-CT/sCT ratio were calculated. Receiver operating characteristic (ROC) analysis was performed to determine the absolute cut-off levels of CT in the washing fluid and the cut-off ratio between FNAB-CT and sCT with the highest sensitivity and accuracy. Diagnostic accuracies of the established cut-offs were compared with that of FNAB-C.

Results

A series of 38 patients with suspicious primary and/or recurrent/metastatic MTC were included. There were 22 females and 16 males, with a mean age of 54.78 ± 15.01 years (range 17-90). FNAB-C, FNAB-CT and FNAB-CT/sCT ratio was performed on 62 thyroid/neck masses or neck lymph nodes. Overall, 20 patients (32%) were evaluated during post-surgical follow-up after primary surgery for MTC.

The mean value of sCT in all patients was 217.48 ± 599.11 pg/ml (range: 3.00-3110.00). The mean lesion size was 13.98 ± 8.22 mm (range: 3.9-30.9) and 14.05 ± 7.99 mm (range: 6-42.3), respectively, for thyroid/neck masses and neck lymph nodes.

Final histological examination confirmed a diagnosis of MTC in 18 lesions in 15 patients. FNAB-C was positive in 9 cases (14.5%) and negative in the remaining 53 cases (85.5%) (Table I).

ROC curve analysis for FNAB-CT showed an area under the curve (AUC) = 99% (p < 0.0001); on the basis of this curve, the more accurate FNAB-CT cut-off was 10.4 pg/ml leading to a sensitivity of 89% and a specificity of 100% (Fig. 1). ROC curve analysis for FNAB-CT/sCT ratio showed an AUC = 90% (p < 0.0001); on the basis of this curve, the more accurate FNAB-CT cut-off was 1.39 leading to a sensitivity of 83% and a specificity of 93% (Fig. 2).

According to this cut-off, FNAB-CT results were considered positive in 16 cases (25.8%) and negative in 46 cases (74.2%). Similarly, the FNAB-CT/sCT ratio results were considered positive in 15 cases (24.1%) and negative in 41 cases (66.1%) (Table I).

The mean value of FNAB-CT in cases with histological-proven MTC was 1085.19 ± 903.96 pg/ml (range 6.98-2000), which was significantly higher than in non-MTC cases (3.96 ± 1.76 pg/ml, range: 1.64-10.40) (p < 0.0001). FNAB-C correctly identified 9 cases with MTC (TP results) and 44 cases without (TN results). FN results were observed in 9 cases, but no FP results were observed. FNAB-C had a sensitivity of 50%, a specificity of 100%,

Table I. Results of FNAB-C*, FNAB-CT†, FNAB-CT†/sCT‡ ratio, and integration of FNAB-C* with FNAB-CT† and FNAB-CT†/sCT‡ ratio compared with histology.

	Positive for MTC¶ (N)	Negative for MTC¶ (N)
FNAB-C*	9	53
FNAB-CT†	16	46
FNAB-CT†/sCT‡ ratio	15	41
FNAB-C* + FNAB-CT†	17	44
FNAB-C* + FNAB-CT†/sCT‡ ratio	16	40
HISTOLOGY	**18**	**44**

** FNAB-C: ultrasound-guided fine-needle aspiration biopsy cytology; † FNAB-CT: calcitonin in the needle wash-out; ‡ sCT: serum calcitonin; ¶ MTC: medullary thyroid carcinoma.*

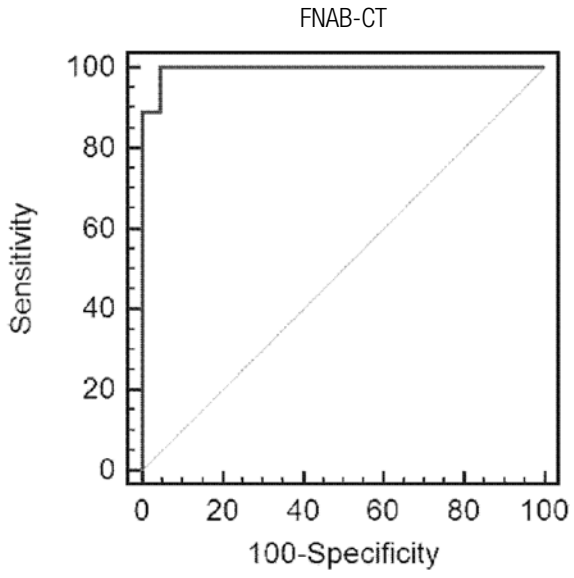
an overall accuracy of 85%, a PPV of 100% and a NPV of 83% (Table II).

FNAB-CT correctly identified 16 cases with MTC (TP results) and 44 cases without (TN results). FN results were observed in 2 cases, but no FP results were reported. For FNAB-CT sensitivity was 89%, specificity 100%, accuracy 97%, PPV 100% and NPV 96% (Table II).

FNAB-CT/sCT ratio correctly identified 15 cases with MTC disease (TP results) and 41 cases without (TN results). FN results were observed in 3 cases; FP results in 3 cases. For the FNAB-CT/sCT ratio sensibility was 83%, specificity 93%, accuracy 90%, PPV 83% and NPV 93% (Table II).

One of 2 patients with a false negative FNAB-CT result and one of 3 patients with a false negative FNAB CT/sCT ratio were correctly diagnosed by FNAB-C. Eight of 9 non-diagnostic FNAB-C were correctly classified by FNAB-CT and 7 by FNAB CT/sCT ratio.

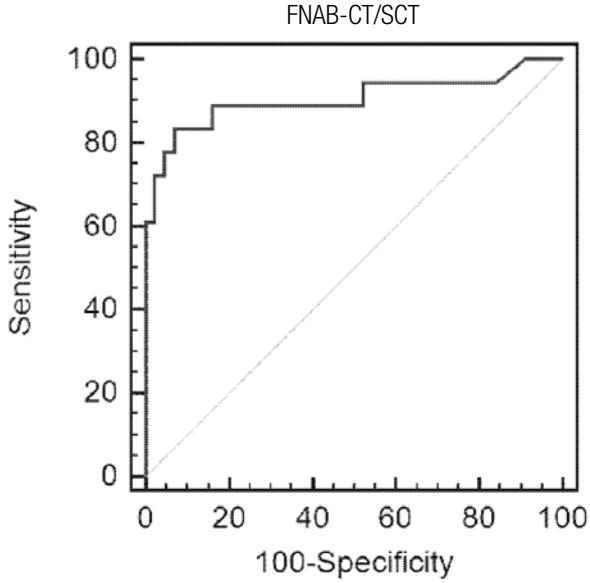
The integration of FNAB-C and FNAB-CT correctly identified 17 cases with MTC (TP results) and 44 cases without (TN results). The FN result was observed in 1 case, but no FP results were reported. The integration of both methods had a sensibility of 94%, a specificity of 100%, an overall accuracy of 98%, a PPV of 100% and a NPV of 98%. The integration of FNAB-C and FNAB-CT/sCT ratio correctly identified 16 cases with MTC disease (TP results) and 40 cases without (TN results). FN results were observed in 2 cases; FP results were observed in 4 cases. The integration of both methods had a sensitivity of 89%, a specificity of 91%, an overall accuracy of 90%, a PPV of 80% and a NPV of 95% (Table II).



*Cut-off = 10.4 pg/ml; sensitivity 89%; specificity 100%; PPV‡ 100%; NPV¶ 96%; accuracy 97%; AUC** = 0.99 (CI†† = 95%, 0.93 - 1.00).*

** ROC: Receiver Operating Characteristic; † FNAB-CT: calcitonin in the needle wash-out; ‡ PPV: positive predictive value; ¶ NPV: negative predictive value; ** AUC: area under curve; †† CI: confidence interval*

Fig. 1. ROC* curve of FNAB-CT†.



Cut-off ratio = 1.39; sensitivity 83%; specificity 93%; PPV¶ 83%; NPV 93%; accuracy 90%; AUC†† = 0.90 (CI‡‡ = 95%, 0.80 - 0.96).*

** ROC: Receiver Operating Characteristic; † FNAB-CT: calcitonin in the needle wash-out; ‡ sCT: serum calcitonin; ¶ PPV: positive predictive value; ** NPV: negative predictive value; †† AUC: area under curve; ‡‡ CI: confidence interval.*

Fig. 2. ROC* curve of the FNAB-CT†/sCT‡ ratio.

Table II. Accuracy, positive predictive value and negative predictive value of FNAB-C*, FNAB-CT†, FNAB-CT†/sCT¶ ratio, and integration of FNAB-C* with FNAB-CT† and FNAB-CT†/sCT¶.

	Sensitivity	Specificity	Accuracy	Positive predictive value	Negative predictive value
FNAB-C*	50%	100%	85%	100%	83%
FNAB-CT†	89%	100%	97%	100%	96%
FNAB-CT†/sCT¶ ratio	83%	93%	90%	83%	93%
FNAB-C* + FNAB-CT†	94%	100%	98%	100%	98%
FNAB-C* + FNAB-CT†/sCT¶	89%	91%	90%	80%	95%

** FNAB-C: ultrasound-guided fine-needle aspiration biopsy cytology; † FNAB-CT: calcitonin in the needle wash-out; ¶ sCT: serum calcitonin.*

Discussion

The early clinical detection and preoperative confirmation of MTC may represent a diagnostic challenge in clinical practice [23]. Indeed, sCT is a sensitive diagnostic tool for diagnosis of MTC [1-5], but the actual diagnostic accuracy of this marker and its use as a routine test in clinical practice are still a matter of debate [3 23]. The FNAB-C reveals a diagnostic accuracy for MTC less consistent than for differentiated thyroid carcinoma [11 23], with a reported sensitivity of 45-63%, indicating that misdiagnosis often occurs with this approach [11 23 24].

As a consequence, in several cases diagnosis of MTC is still incidentally made postoperatively with the risk of an incomplete surgical treatment. A tailored approach, including total thyroidectomy plus central neck node dissection [25 26], and lateral neck dissection with therapeutic intent, is advisable for adequate treatment but requires early preoperative diagnosis and correct clinical staging of MTC [23].

Recently, several studies have reported that the measurement of CT in the washout of the FNAB needle identifies MTC with high sensitivity and specificity, indicating that this approach may be a useful adjunct to conventional FNAB-C in patients with increased sCT [12-18].

A similar approach is used to identify neck recurrences/metastases of differentiated papillary or follicular thyroid carcinoma with the measurement of thyroglobulin (Tg) in fine-needle aspiration biopsy washout fluid (FNAB-Tg) [27]. When compared with FNAB-Tg, a smaller number of studies have been performed to evaluate the usefulness of CT assay in FNAB fluid alone or combined with cytology. However, the few papers on this topic have consistently shown that FNAB-CT has high accuracy [23].

Despite the emerging role of FNAB-CT for the diagnosis of primary or metastatic MTC [2], to date, there is no established method for FNAB-CT sampling, or an established cut-off of FNAB-CT for diagnosis of MTC.

Boi et al. [12] proposed an 'arbitrary' FNAB-CT cut-off of 36 pg/ml, corresponding to three times the highest value found in controls. Similarly, Kudo et al. [13] performed the technique in a series of five patients: MTC was detected by FNAB-CT in all cases, while cytology was positive for MTC in one case. Moreover, Abraham et al. [15] reported a series of five MTC patients undergoing FNAB-C on suspicious neck lymph nodes prior to surgery, with an accurate localisation of metastases. Massaro et al. [28] evaluated FNAB-CT in 27 patients, and no MTC was diagnosed. This suggests that more study on larger series are necessary to establish a validated cut-off value for FNAB-CT.

In our series, ROC analysis was performed to determine the absolute cut-off levels of CT in the washing fluid and the cut-off ratio between FNAB-CT and sCT with the highest sensitivity and accuracy: we obtained levels of FNAB-CT > 10.4 pg/ml and FNAB-CT/sCT ratio > 1.39 as the more accurate cut-offs.

FNAB-CT was more accurate than FNAB-C (97% *vs.* 85%), but the integration of both methods had better diagnostic performance (98% of accuracy) than FNAB-CT or FNAB-C alone.

Moreover, a fixed cut-off was not always appropriate, particularly in patients with extremely high sCT, due to peripheral blood contamination of needle wash-out fluid. To better characterise this potential interference, we analysed FNAB-CT related with the sCT by using their ratio [28]. The FNAB-CT/sCT ratio was more accurate than FNAB-C (90% *vs.* 85%), and the integration of both methods had better accuracy than cytology alone (90% *vs.* 85%). Unexpectedly, FNAB-CT had a higher diagnostic performance than FNAB-CT/sCT ratio (97% *vs.* 90%) either alone or integrated with FNAB-C (98% of accuracy for FNAB-CT in combination with FNAB-C *vs.* 90% for the FNAB-CT/sCT ratio in combination with FNAB-C).

On the basis of our findings, it seems clear that FNAB-CT should be integrated, but that it cannot replace or substitute FNAB-C in detection of primary or metastatic MTC. Indeed, FNAB-CT is particularly useful to determine correct diagnosis in the presence of non-diagnostic FNAB-C. Eight of 9 non-diagnostic FNAB-C were correctly classified by FNAB-CT and 7 by FNAB CT/sCT ratio.

On the other hand, it seems obvious that cytological examination cannot be eliminated or replaced, because it is essential for diagnosis, and has very high specificity and sensitivity, particularly when the CT is not conclusive; one of 2 patients with a false negative FNAB-CT result and one of 3 patients with a false negative FNAB CT/sCT

ratio were correctly diagnosed by FNAB-C in our series. As a consequence, FNAB-C should be considered complementary to FNAB-CT, and these complementary methods can be considered as essential in identification of patients with MTC as they can contribute to correct diagnosis and aid in planning appropriate therapy.

Conclusions

FNAB-CT, in addition to cytology, should be considered the standard in pre-surgical diagnostic work-up of MTC and of suspicious neck MTC recurrences/metastases. This may have important implications in the management of MTC. FNAB-CT should integrate but not substitute FNAB-C to detect MTC; it is especially helpful in the case of non-diagnostic FNAB-C.

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