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Calcitonin Measurement in Wash-Out Fluid from Fine Needle Aspiration of Neck Masses in Patients with Primary and Metastatic Medullary Thyroid Carcinoma

Francesco Boi, Ivan Maurelli, Giovanni Pinna, Francesca Atzeni, Mario Piga, Maria Letizia Lai, and Stefano Mariotti

Endocrinology (F.B., I.M., G.P., F.A., S.M.) and Nuclear Medicine (M.P.), Department of Medical Sciences "M. Aresu," and Department of Cytomorphology, San Giovanni di Dio Hospital (M.L.L.), University of Cagliari, I-09042 Monserrato, Cagliari, Italy

Objective: The aim of the study was to evaluate the usefulness of calcitonin (CT) assay in fine-needle aspiration biopsy (FNAB) wash-out fluid alone or combined with cytology in the presurgical study of medullary thyroid carcinoma (MTC) patients with thyroid nodules (TNs) and of suspicious neck MTC recurrences/metastases.

Subjects and Methods: A total of 36 ultrasound-guided FNABs were performed in neck masses from 23 patients with borderline or high basal and pentagastrin-stimulated serum CT. Cytology and CT-FNAB were performed on a total of 18 TNs and three neck lymph nodes (LNs) from 12 patients examined before thyroidectomy, and on six suspicious local recurrences (LRs) and nine LNs from nine totally thyroidectomized MTC patients. On the basis of CT-FNAB values found in 15 non-MTC lesions, CT-FNAB more than 36 pg/ml was considered as indicative of MTC.

Results: All 21 positive CT-FNAB lesions (10 TNs, six LNs, and five LRs), 13 with positive cytology, were confirmed as MTC at histology. Of the 15 negative CT-FNAB suspicious masses (eight TNs, six LNs, and one LR), five displayed a benign lesion at histology. The remaining 10 cases, all with benign cytology, were not operated on, and no evidence of MTC was detected at follow-up. CT-FNAB reached 100% sensitivity and specificity for MTC, while cytology displayed 61.9% sensitivity and 80% specificity.

Conclusions: Ultrasound-guided CT-FNAB was the best tool to identify primary MTC and LRs/node metastases in MTC operated subjects. This may have important implications in the management of MTC. **(*J Clin Endocrinol Metab* 92: 2115–2118, 2007)**

BASAL AND PENTAGASTRIN (PG)-stimulated serum calcitonin (CT) has been proposed as a routine assay in the diagnostic evaluation of all thyroid nodules (TNs) for early detection of medullary thyroid carcinoma (MTC) (1–4). Fine-needle aspiration cytology represents the main tool in the diagnostic evaluation of TNs, but it is not frequently proposed as a routine procedure in patients with high-serum CT, due to its low sensitivity and specificity (1, 5). Neck ultrasound (US) with color flow Doppler sonography (CFDS) and US-guided fine-needle aspiration cytology is the current diagnostic approach for the identification of MTC neck lymph node (LN) metastases both before and after thyroidectomy (6–8). A similar approach is used to identify neck recurrences/metastases of differentiated follicular and papillary thyroid carcinoma, where the assay of thyroglobulin (Tg) in fine-needle aspiration biopsy (FNAB) wash-out fluid (Tg-FNAB) represents an additional highly reliable marker (9–11). When compared with Tg-FNAB, no study has been performed to evaluate the usefulness of CT assay in FNAB (CT-FNAB) fluid alone or combined with cytology.

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Abbreviations: CFDS, Color flow Doppler sonography; CT, calcitonin; CT-FNAB, calcitonin assay in FNAB; FNAB, fine-needle aspiration biopsy; LN, lymph node; MTC, medullary thyroid carcinoma; PG, pentagastrin; Tg, thyroglobulin; TN, thyroid nodule; US, ultrasound.

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The aim of this study was to assess the clinical usefulness of CT-FNAB in the identification of primary MTC and its neck LN metastases or recurrences.

Patients and Methods

Patients studied

From 2003–2006, 36 US-guided FNABs were performed in 23 patients with high-serum CT. These included 21 lesions (18 TNs and three neck LNs) from 14 patients before thyroidectomy and 15 neck masses (six suspicious local recurrences and nine neck LNs) from nine patients after total thyroidectomy for MTC.

All patients (13 women, age range 36–76 yr, and 10 men, age range 25–69 yr) were submitted to an accurate physical examination, neck US, US-guided FNAB of neck mass for conventional cytology, and CT-FNAB. Basal serum and PG-stimulated CT were performed as detailed later. Thyroid ^{99m}Tc-pertechnetate scintiscan was also performed in most patients with TNs before surgery.

Conventional neck US and CFDS study

US and CFDS of the neck were performed using Sonoline G60S equipment (Siemens, Medical Solutions, Issaquah, WA) with a 8–13-mHz linear electronic transducer. All TNs and cervical masses were identified and localized, and their diameters were measured. Suspicious TNs and neck masses were identified according to standard criteria (6, 11) and submitted to FNAB under US visual control.

Hormonal assays

Serum CT and CT-FNAB measurements were performed using an ultrasensitive chemiluminescent assay (Immulite 2000 Calcitonin; Diagnostic Products Corp., Los Angeles, CA, distributed by Medical Systems Corp., Genoa, Italy). Normal range values for serum CT were less

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than 1–18 pg/ml for males and less than 1–12 pg/ml for females. Unless very elevated (>500 pg/ml), serum CT was assayed before and after stimulation with PG. For this purpose CT was assayed before, and two and 5 min after iv bolus of 0.5 μg/kg PG (Pentagastrin Injection BP; Cambridge Laboratories, Tyne and Wear, UK). Serum CT after PG stimulation was considered abnormal if more than 100 pg/ml (12).

Cytology and CT-FNAB

Written informed consent for the study was obtained before FNAB. US-guided FNAB was performed as previously reported (11, 13). Cytological examination was made by an experienced thyroid pathologist (M.L.L.), who was unaware of CT-FNAB results. Cytological diagnosis in TNs and LNs was expressed according to standard criteria, as previously detailed (11, 13). A specific diagnosis of MTC was attempted in all case when cytological features suggestive for this tumor were found (14). For the purpose of this study, cytology was considered "positive" when suggestive of malignant neoplasm with or without specific MTC characteristics and "negative" in the presence of benign pattern or inadequate sample.

After smear preparation, the needle was washed out with 500 μl CT-free serum dilution buffer and the solution processed for CT-FNAB measurement.

Histological diagnosis on surgical specimens was made using standard pathological techniques, including search of CT expression by immunohistochemistry.

Cutoff values for CT-FNAB

To interpret correctly the results of CT-FNAB, we had to establish a cutoff above which the CT-FNAB concentration in wash-out fluid could be considered expression of local CT production, rather than the result of peripheral blood contamination. For this purpose we retrospectively examined all CT-FNABs obtained from benign TNs and from non-MTC cervical masses of patients with borderline to markedly increased serum CT levels. On the basis of this analysis, all CT-FNABs more than 36 pg/ml (*i.e.* three times the maximal CT-FNAB concentration found in this group) were considered positive and diagnostic for MTC.

Statistical analysis

The sensitivity and specificity for primary MTC and MTC recurrences/LNs metastases were calculated by the Galen and Gambino formula (15).

Results

The results of CT-FNAB are shown in Fig. 1A. There were 18 TNs (14 patients) submitted to FNAB before thyroidectomy. CT-FNAB was clearly positive (860 to >2000 pg/ml) in 10 nodules, seven of which were also positive for MTC at cytology. Basal (21–2000 pg/ml) and PG-stimulated (227–1521 pg/ml) serum CT concentration was increased in all cases. Histological examination confirmed MTC in all cases. Eight nodules had both negative CT-FNAB and cytology. Of them, four nodules, all with low but detectable CT-FNAB (4–12 pg/ml), were histologically proven benign thyroid lesions associated with a concomitant MTC. The other four nodules, all with undetectable (<1 pg/ml) CT-FNAB, were from patients with borderline increase of basal serum CT (16–40 pg/ml), without a significant increase (76–88 pg/ml) after PG stimulation. These patients were not operated on, and no evidence of MTC was detected during 1.5–2.5-yr follow-up. Basal and PG-stimulated CT remained in the same range (17–25 pg/ml basal and 78–90 pg/ml stimulated) when retested (one to two times).

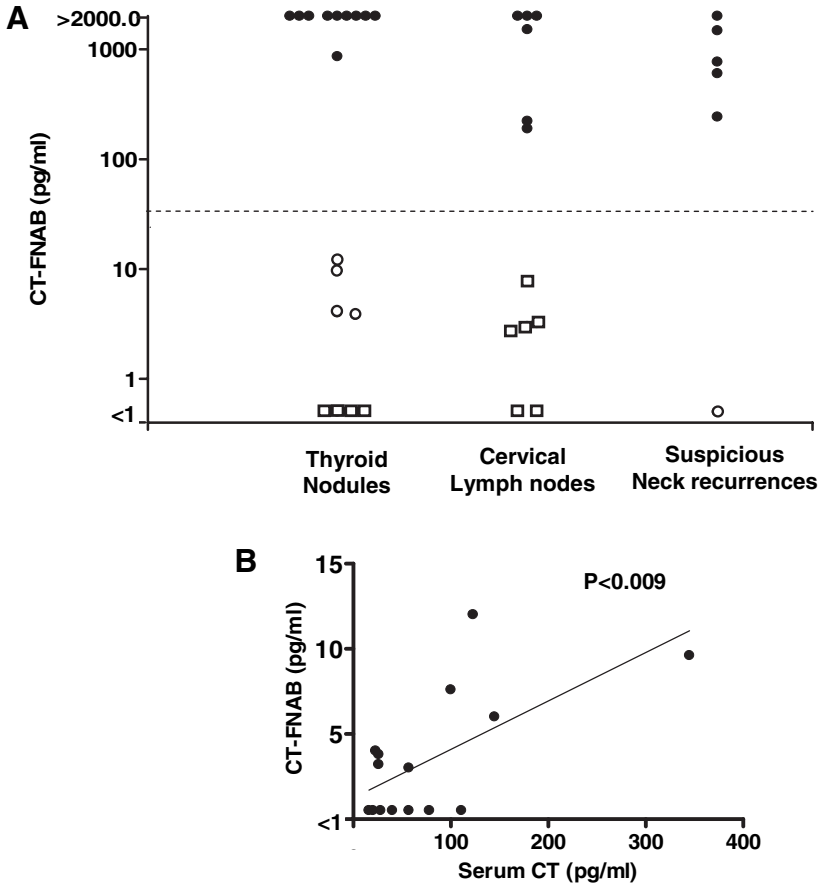

FIG. 1. A, CT-FNAB concentrations found in histologically proven MTC neck lesions (●), histologically proven benign lesions (○), and nonoperated TNs and cervical LNs that did not show any progression when followed up to 2.5 yr (□). The *dotted line* indicates the cutoff value of 36 pg/ml. B, Correlation between serum CT and CT-FNAB values found in benign neck lesions.

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A total of 12 cervical LNs were submitted to FNAB. Six nodes, all with clearly positive CT-FNAB (189 to >2000 pg/ml), were surgically removed, and in all cases a MTC metastasis was confirmed at histology. Cytology was positive for malignant neoplasia only two cases, the remaining four being not diagnostic. Three of these nodes were from one of the 14 patients evaluated preoperatively with a TN-harboring MTC. The remaining CT-FNAB positive nodes were from three patients previously thyroidectomized for MTC with persistent increased basal (550 to >2000 pg/ml) serum CT concentration. Negative CT-FNAB (<1.0–7.6 pg/ml) and cytology were found in another six LNs with dubious clinical and US features of four previously thyroidectomized MTC patients with persistent high basal (26–111 pg/ml) and PG-stimulated (1275 to >2000 pg/ml) serum CT. These patients were not operated on and were followed (2–3 yr) by periodic US examination, showing in all cases a reduction of the node size, further supporting their nonneoplastic (reactive) nature. FNAB was also performed on six neck masses from five MTC patients with suspicious local recurrences after total thyroidectomy and increased basal (78 to >2000 pg/ml) and/or PG-stimulated (470–1508 pg/ml) serum CT. Five CT-FNAB positive cases (242 to >2000 pg/ml, four positive also for cytology) were operated on, and a recurrence of MTC was histologically confirmed in all. One patient also had a second suspicious mass, with indeterminate cytology and negative CT-FNAB (<1.0 pg/ml); this lesion was surgically removed and resulted in an inflammatory granuloma at histology.

Together, high CT-FNAB concentrations (189 to >2000 pg/ml) were found in all histologically confirmed MTCs. In contrast, CT-FNAB in benign lesions was often undetectable (<1 pg/ml), and, in any case, not more than 12 pg/ml. When detectable, no difference was found between CT-FNAB from benign TNs and nonneoplastic neck masses (Fig. 1A). To evaluate whether and to what extent the increased serum CT concentration could contribute to CT-FNAB, serum CT was compared with CT-FNAB of benign lesions (Fig. 1B). A significant correlation between serum CT and CT-FNAB was found ($r = 0.642$; $P < 0.009$); the 95% confidence interval of the regression line slope was 0.008–0.049, indicating that the contribution of serum CT in CT-FNAB should correspond to about 0.8–5% of serum CT.

The comparison of CT-FNAB with cytology and histology is shown in Table 1. CT-FNAB was able to detect all 21 histologically proven primary or recurrent/metastatic MTCs, while cytology correctly identified only 13 cases (61.9%). On the other hand, none of the 15 lesions with negative CT-FNAB was MTC, as assessed by histology in five operated cases, and by clinical and US follow-up in the remaining 10 lesions (four TNs and six LNs). The diagnostic accuracy for MTC of cytology and CT-FNAB was calculated in the 26 operated lesions. CT-FNAB reached 100% sensitivity and specificity, while cytology displayed only 61.9% sensitivity and 80% specificity. False-negative cytological results (8 of 21, 38.1%) were mostly due to inadequate or insufficient material (7 of 8, 87.5%), while only in one case the diagnosis was "indeterminate follicular lesion."

TABLE 1. CT-FNAB results compared with cytology and histology

	Positive CT-FNAB fluid (n = 21)	Negative CT-FNAB fluid (n = 15)
Cytology for MTC		
No. of positive	13	1
No. of negative	8	14[a]
Histology for MTC		
No. of positive	21	0
No. of negative	0	5

[a] There were 10 lesions not operated on that displayed benign clinical features at follow-up.

Discussion

Serum CT is the most sensitive diagnostic tool for MTC, but it is not helpful to localize primary tumors in the thyroid gland and its neck recurrences/metastases in thyroidectomized patients. In this study we assessed the potential usefulness of CT-FNAB alone or combined with cytology, in localization of primary or metastatic MTC, following the model of Tg-FNAB (9–11). For this purpose we retrospectively examined 36 consecutive US-guided FNABs performed in TNs and selected neck masses of patients with increased basal and/or PG-stimulated serum CT evaluated for primary or recurrent MTC. The results obtained showed that high-CT concentrations were present in the FNAB needle washout in all histologically proven MTC, either in thyroid gland or neck masses. CT-FNAB reached 100% sensitivity and specificity when all data were taken together, while cytology displayed lower diagnostic accuracy (61.9% sensitivity and 80% specificity), a value similar to that reported in previous studies (1, 5, 7).

The above excellent diagnostic performance of CT-FNAB was obtained with a rather arbitrary cutoff of 36 pg/ml corresponding to three times the highest CT-FNAB concentration observed in our series of benign cervical lesions. However, we are aware that a fixed cutoff could not be always appropriate, particularly in patients with extremely high-serum CT, due to peripheral blood contamination of needle wash-out fluid. To characterize better this potential interference, we analyzed CT-FNAB concentrations found in benign neck masses from patients with increased serum CT. The results obtained, although limited by the small number of cases, strongly suggest that serum CT contribution to CT-FNAB does not exceed 5%, a value that could not account for any of the positive CT-FNABs found in this study. CT-FNABs in neck LN metastases or recurrences were not much higher than the corresponding serum CT, a finding in contrast with what was generally observed with Tg-FNAB (9–11). This may be due to differences in the respective amounts of Tg and CT within the metastatic cells or released into the interstitial fluid during needle aspiration.

In conclusion, our study provides the first demonstration that CT-FNAB is a highly reliable diagnostic procedure to identify primary and recurrent/metastatic MTC. The actual relevance of this technique in the management of MTC needs further longitudinal studies in a larger number of patients.

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Address all correspondence and requests for reprints to: Stefano Mariotti, M.D., Endocrinology, Department of Medical Sciences, Presidio di Monserrato, University of Cagliari, Strada Statale 554-bivio Sestu, I-09042 Monserrato, Cagliari, Italy. E-mail: mariotti@pacs.unica.it.

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