J Clin Endocrin Metab. First published ahead of print June 9, 2010 as doi:10.1210/jc.2010-0045

A Decrease of Calcitonin Serum Concentrations Less Than 50 Percent 30 Minutes after Thyroid Surgery Suggests Incomplete C-Cell Tumor Tissue Removal

A. Faggiano, F. Milone, V. Ramundo, M. G. Chiofalo, I. Ventre, R. Giannattasio, R. Severino, G. Lombardi, A. Colao, and L. Pezzullo

Istituto di Ricovero e Cura a Carattere Scientifico Fondazione SDN (A.F.), and Nuclear Medicine Unit (R.G.), Azienda Sanitaria Locale Napoli 1 Centro, 80143 Napoli, Italy; Department of Molecular and Clinical Endocrinology and Oncology (F.M., V.R., R.S., G.L., A.C.), Federico II University, and Surgery Unit (M.G.C., L.P.), National Cancer Institute, Fondazione G. Pascale, 80131 Napoli, Italy; and Endocrinology Unit (I.V.), G. Rummo Hospital, 80121 Benevento, Italy

Context and Objectives: The prognosis of medullary thyroid carcinoma (MTC) depends on the completeness of the first surgical treatment. To date, it is not possible to predict whether the tumor has been completely removed after surgery. The aim of this study was to evaluate the reliability of an intraoperative calcitonin monitoring as a predictor of the final outcome after surgery in patients with MTC.

Patients and Methods: Twenty patients underwent total thyroidectomy and central lymph node dissection on the basis of a positive pentagastrin test. In six cases a preoperative diagnosis of MTC was achieved at the cytological examination. During the surgical intervention, calcitonin was measured at the time of anesthesia, at the time of manipulation, and 10 and 30 min after surgical excision. At the histological examination, 10 patients had MTC and 10 had C cell hyperplasia.

Results: As compared with calcitonin levels before thyroidectomy, a decrease of calcitonin greater than 50% 30 min after surgery was able to significantly distinguish patients who were cured from those who experienced persistence of disease. It was not possible to find a similar result when the decrease of calcitonin 10 min after surgery was considered.

Conclusions: A rate of calcitonin decrease less than 50% 30 min after thyroidectomy plus central neck lymph node dissection suggests the persistence of tumor tissue in patients operated for MTC. These results indicate that intraoperative calcitonin monitoring may be a useful tool to predict the completeness of surgery in patients with MTC. (***J Clin Endocrinol Metab*** **95: 0000–0000, 2010)**

Medullary thyroid carcinoma (MTC) originates from the parafollicular cells (C cells) of the thyroid. These cells produce calcitonin, which is a highly sensitive and specific marker of MTC for both diagnosis and follow-up (1, 2). The definitive cure of the tumor is strongly dependent on the completeness of the first surgical treatment (3). If total thyroidectomy and central neck lymph node dissection are performed in all cases of MTC, it is a matter of debate whether to perform lateral lymph node dissection in any case or to limit this radical approach only to patients with metastases in the central neck compartment or palpable lateral neck lymph nodes (4–8). For this purpose, it should be considered that the risk of metastases in the contralateral neck compartment and the mediastinum depends on the size of the primary tumor, the number of central lymph node metastases, and tumor multicentricity (8–10). If the first surgical treatment is so crucial for the final outcome of the patient, on the other hand, there are no clear criteria that can indicate to the surgeon whether the tumor has been completely removed after to-

ISSN Print 0021-972X ISSN Online 1945-7197
Printed in U.S.A.

doi: 10.1210/jc.2010-0045 Received January 7, 2010. Accepted May 21, 2010.

Abbreviations: CCH, C cell hyperplasia; MTC, medullary thyroid carcinoma; PG, pentagastrin; US, ultrasonography.

tal thyroidectomy plus central lymph node dissection. In a recent study evaluating the usefulness of intraoperative pentagastrin (PG) test in predicting lymph node involvement in patients with MTC who underwent total thyroidectomy plus central lymph node dissection, 80% of subjects were correctly recognized to have postoperative tumor remnants, and this procedure failed to detect tumor remnant in 20% of uncured subjects (11). In the present study, intraoperative calcitonin monitoring was performed in patients who were recommended for surgery on the basis of the detection of high basal and PG-stimulated calcitonin levels consistent with a diagnosis of MTC to evaluate the reliability of this intraoperative procedure in predicting the completeness of surgery.

Patients and Methods

Patients

Twenty consecutive patients (nine males and 11 females, aged 25–70 yr, range 50 ± 2.3 yr), candidates for thyroid surgery on the basis of abnormally increased calcitonin levels and without preoperative evidence of lymph node or distant metastases, were enrolled in the study. All patients underwent thyroid surgery on the basis of basal or PG calcitonin concentrations above 100 ng/liter (12). Five patients had basal serum calcitonin concentrations greater than 100 ng/liter, and 15 other patients had basal calcitonin concentrations between 18 and 68 ng/liter and PG-stimulated calcitonin concentrations greater than 100 ng/liter. All patients had thyroid nodules at ultrasonography (US): eight patients had a single nodule and 12 patients had two or more nodules. None of the patients had positive lymph nodes on preoperative US.

A fine-needle aspiration biopsy was performed under US guidance in all patients on the thyroid nodules with US features suspicious for malignancy (absence of halo sign, microcalcifications. and hypoechogenicity) (13) or, in absence of this condition, on the largest nodule: a preoperative diagnosis of MTC was obtained at fine-needle aspiration biopsy in six of 20 patients. None of the patients was positive at the genetic analysis of *RET*. The surgical procedure for all of the patients consisted in total thyroidectomy and central compartment lymph node dissection. The latter consisted in the removal of the contents of the pretracheal and paratracheal compartments bilaterally. Because none of the patients had lymph node metastases at the preoperative US examination or at the intraoperative surgical exploration, a radical neck dissection, including both central and bilateral neck lymph node dissection, was not performed in any patient.

At the histological examination, MTC was found in 10 patients and C cell hyperplasia (CCH) only in 10 other patients (Table 1). All patients gave written informed consent. The study was conducted in accordance with the Declaration of Helsinki, adhering to all local regulatory guidelines.

Outcome

After surgery, calcitonin was measured every 3 months during the first year and then every 6 months; a PG test was performed 6 months after surgery in those patients with a histological diagnosis of MTC. A color-Doppler US was performed every 6 months in all patients. The mean follow-up time after surgery was 14.6 ± 1.7 months (range 6–30 months). There was no evidence of disease persistence or relapse in 16 of the 20 patients. Disease persistence was found in four patients, two of them with both basal and PG-stimulated abnormally high calcitonin levels and two other patients with basal calcitonin levels 5 ng/liter or less but a progressive increase of calcitonin during the follow-up and positive PG-stimulated calcitonin response. These patients underwent a careful radiological work-up and were followed up periodically with color Doppler US, contrast-enhanced computed tomography and magnetic resonance imaging scan, and ^{18}F-deoxy-D-glucose-positron emission tomography. One of the four patients with persistent disease after surgery had US evidence of tumor remnant in neck lymph node, which was confirmed at the ^{18}F-deoxy-D-glucose-positron emission tomography scan. This patient underwent a second surgery to remove bilateral neck lymph nodes. The other three patients with postoperative positive calcitonin did not show radiological signs of tumor at the last radiological examination performed 12, 16, and 22 months after surgery, respectively.

TABLE 1. Patient characteristics

n	Age/sex	Basal calcitonin (ng/liter) before surgery	Histology	Tumor stage
1	50/Female	68	MTC	T1aN0
2	42/Female	198	MTC	T1aN0
3	49/Female	42	MTC	T1aN0
4	70/Female	465	MTC	T2aN1
5	37/Male	29	CCH	NA
6	58/Female	240	MTC	T1aN0
7	25/Male	20	CCH	NA
8	57/Male	55	CCH	NA
9	48/Male	108	MTC	T2aN1
10	32/Female	20	CCH	NA
11	59/Male	23	CCH	NA
12	47/Male	25	CCH	NA
13	55/Male	18	CCH	NA
14	58/Female	107	MTC	T1aN0
15	45/Male	31	CCH	NA
16	56/Male	45	MTC	T2aN0
17	49/Female	56	MTC	T2aN0
18	51/Female	28	CCH	NA
19	54/Female	67	MTC	T1aN0
20	59/Female	32	CCH	NA

NA, Not applicable.

Calcitonin assay

Serum calcitonin concentrations were determined by a commercially available two-site sandwich-type chemiluminescent immunometric assay (DiaSorin Inc., Stillwater, MN). Normal values were 0.4–18.9 ng/liter for males and 0.0–5.5 ng/liter for females. Analytic sensitivity was 1 ng/liter or less. The intra- and interassay coefficients of variation were 3 and 4%, respectively, at 70.4 ng/liter and 1 and 2%, respectively, at 179 ng/liter. No hook effect was observed until a calcitonin concentration of 500,000 ng/liter. The calcitonin assay run time was 15 min.

PG + calcium test was performed as follows: calcium was injected first, iv in 1 min at a dose of 2 mg/kg. PG was injected in 15 sec at a dose of 0.5 μg/kg in 5 ml of NaCl solution 0.9%.

TABLE 2. Calcitonin decrease and outcome after surgery

n	Age/Sex	Calcitonin reduction 10 min after tumor removal (%)	Calcitonin reduction 30 min after tumor removal (%)	Outcome	Follow-up (months)	Calcitonin levels (ng/liter) at the last follow-up time
1	50/Female	71	72	Remission	26	<2
2	42/Female	15	63	Remission	18	<2
3	49/Female	35	61	Remission	6	5
4	70/Female	69	16	Persistence	24	380
5	37/Male	58	67	Remission	12	5
6	58/Female	74	79	Remission	8	12
7	25/Male	70	65	Remission	6	4
8	57/Male	25	55	Remission	10	<2
9	48/Male	56	39	Persistence	16	285
10	32/Female	13	56	Remission	12	<2
11	59/Male	76	80	Remission	6	5
12	47/Male	36	55	Remission	8	<2
13	55/Male	54	77	Remission	6	<2
14	58/Female	21	56	Remission	30	<2
15	45/Male	28	69	Remission	18	9
16	56/Male	30	40	Persistence	19	194
17	49/Female	29	44	Persistence	9	155
18	51/Female	41	71	Remission	16	5
19	54/Female	45	77	Remission	22	<2
20	59/Female	33	59	Remission	21	<2

Blood samples were collected at 0, 1, 2, 3, 5, and 10 min after the injection. According to Costante *et al.* (12), calcitonin peak after a PG test greater than 100 pg/ml was considered indicative for MTC.

The intraoperative calcitonin monitoring procedure included a first calcitonin measurement at the time of anesthesia (preincision value); the following steps were measurement at the time of manipulation (manipulation value), 10 min after surgical excision and 30 min after surgical excision. It was considered the percent reduction from the highest preexcision value, using the highest value between the preincision or the manipulation value. Blood sampling site was an antecubital vein.

Statistical analysis

The statistical analysis was performed by SPSS for Windows version 10 (SPSS, Inc., Chicago, IL). Data were expressed as mean ± SEM. The significance was defined as $P < 0.05$. The percent calcitonin decrease after the surgical intervention was analyzed by receiver operator characteristic analysis by using a non parametric model to determine the best cutoff to distinguish patients cured and uncured by surgery.

Results

At the histological examination, 10 patients were affected with MTC and 10 patients with CCH. Among patients with MTC, tumor stage was T1N0 in six, T2N0 in two, and T2N1 in two (Table 1). In the whole population, preexcision values of serum calcitonin were 908 ± 695 ng/liter (range 11–9160 ng/liter) and postexcision values were 319 ± 202 ng/liter (range 9–2845 ng/liter) 10 min after the surgical excision and 146 ± 75 ng/liter (range 7–1065 ng/liter) 30 min after the surgical excision. The mean percentage decrease was 44 ± 4.6% (range 13–76%) 10 min after surgery and 61 ± 2.9% (range 39–80%) 30 min after surgery (Table 2 and Fig. 1).

At the receiver operator characteristic analysis, a decrease of calcitonin serum concentrations greater than 50% 30 min after surgery was able to significantly distinguish between patients who were cured and those who had

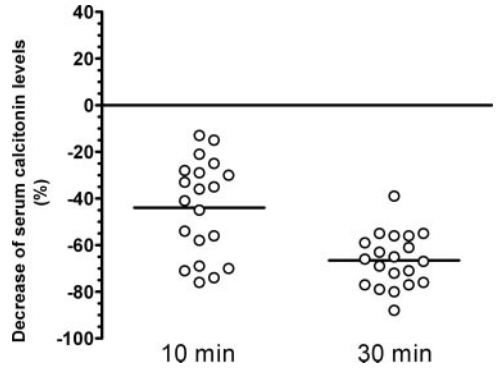

FIG. 1. Percent calcitonin decrease 10 and 30 min after surgery.

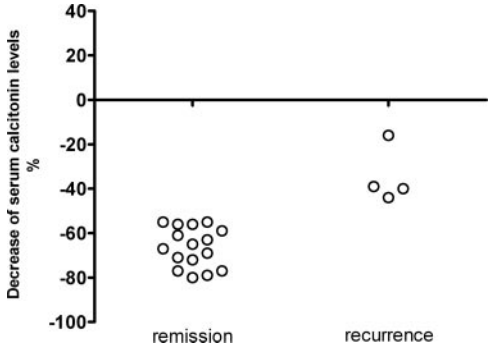

FIG. 2. Correlation between tumor outcome and percent calcitonin decrease 30 min after surgery.

disease persistence during the follow-up with 100% sensitivity and specificity ($P < 0.05$) (Fig. 2). No statistical significance was found to predict the patient outcome when considering calcitonin serum concentrations 10 min after surgery.

An intraoperative calcitonin decrease greater than 50% 30 min after the surgical resection was achieved in all the 16 patients who had complete remission during the follow-up (66 ± 2.4%), whereas the calcitonin decrease after 30 min was 16, 39, 40, and 44%, respectively, in the four patients who had persistent disease after surgery (Fig. 2).

Discussion

The prognosis of a patient suffering from MTC is strongly related to the completeness of the first surgical intervention. If tumor tissue is not totally removed, the subsequent surgery is not as effective as a complete primary surgery in achieving the disease remission (14).

Although preoperative calcitonin concentrations have been reported to predict calcitonin normalization after surgery (12), no clinical or biological parameters clearly predict the postoperative outcome of MTC patients.

The main message of this study is that the intraoperative calcitonin monitoring seems to represent a reliable procedure to determine, at the time of surgery, whether the patient can be considered free of disease after a conservative surgery limited to total thyroidectomy and central compartment lymph node dissection. In the presence of a calcitonin decrease greater than 50% 30 min after surgery, tumor removal can be considered complete. On the other hand, a calcitonin decrease less than 50% 30 min after surgery indicates an incomplete tumor removal and suggests to the surgeon to extend the operation on other lymph node compartments. In their study, Scheuba *et al.* (11) tried to validate a similar procedure, measuring the response to intraoperative PG test to predict lateral lymph node involvement and, consequently, whether to extend surgery to other lymph node compartments after central dissection. Although both the study by Scheuba *et al.* and our study used a chemiluminescent method for calcitonin measurement, a comparison between these two studies is not completely feasible due to a different interassay coefficient of variation. Apart from these technical aspects, the intraoperative calcitonin monitoring seems to be more cost effective than the intraoperative PG test in recognizing patients with incomplete surgery. The main difference is that the sensitivity and negative predictive value of the intraoperative calcitonin monitoring was 100% without any false-negative result, whereas the sensitivity of intraoperative PG test was 80% and negative predictive value 91% (11). This means that the latter procedure did not recognize 20% of patients who had persistent disease after surgery. A second relevant aspect is that the PG test is a much more expensive procedure than not stimulated calcitonin levels measurement and furthermore PG is not commercially available in some countries.

By using a chemiluminescent assay, as performed in the current study, calcitonin measurement takes no longer than 15 min. This makes this technique suitable for an intraoperatory application, as already performed in intraoperative PTH monitoring in patients with primary hyperparathyroidism, in which the chemiluminescent assays show to be the best method for measuring PTH (15), requiring approximately 12 min and clearly indicating to the surgeon before the end of surgery whether the source of PTH has been removed. Furthermore, in comparison with ELISA, chemiluminescent assays show to have advantages in handling a lower detection limit and a higher sensitivity (16).

In conclusion, these results highlight that a decrease of calcitonin serum concentrations greater than 50% 30 min after surgery indicates that all the calcitonin-producing tumor tissue has been removed. On the contrary, a calcitonin decrease lower than 50% seems to suggest an incomplete surgery and, of consequence, a persistence of disease. By a practical point of view, the intraoperative calcitonin monitoring may reliably indicate whether MTC patients had to be treated with a radical lymph node dissection.

Acknowledgments

Address all correspondence and requests for reprints to: Antongiulio Faggiano, M.D., Ph.D., Istituto di Ricovero e Cura a Carattere Scientifico Fondazione SDN, Via E. Gianturco 113, 80143 Napoli, Italy. E-mail: afaggian@unina.it; or afaggiano@hotmail.com.

Disclosure Summary: The authors have nothing to disclose.

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