

ORIGINAL ARTICLE

Prophylactic Thyroidectomy in Multiple Endocrine Neoplasia Type 2A

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ABSTRACT

BACKGROUND

Medullary thyroid carcinoma is the most common cause of death in patients with multiple endocrine neoplasia (MEN) type 2A (MEN-2A) or type 2B or familial medullary thyroid carcinoma. We sought to determine whether total thyroidectomy in asymptomatic young members of kindreds with MEN-2A who had a mutated allele of the *RET* proto-oncogene could prevent or cure medullary thyroid carcinoma.

METHODS

A total of 50 patients 19 years of age or younger who were consecutively identified through a genetic screening program as carriers of a *RET* mutation characteristic of MEN-2A underwent total thyroidectomy. Five to 10 years after the surgery, each patient was evaluated by physical examination and by determination of plasma calcitonin levels after stimulation with provocative agents.

RESULTS

In 44 of the 50 patients, basal and stimulated plasma calcitonin levels were at or below the limits of detection of the assay (proportion, 0.88; 95 percent confidence interval, 0.76 to 0.95). Two patients had basal and stimulated plasma calcitonin levels above the normal range. Stimulated plasma calcitonin levels had increased but remained within the normal range in four patients. The data suggest that there was a lower incidence of persistent or recurrent disease in children who underwent total thyroidectomy before eight years of age and in children in whom there were no metastases to cervical lymph nodes.

CONCLUSIONS

In this study, young patients identified by direct DNA analysis as carriers of a *RET* mutation characteristic of MEN-2A had no evidence of persistent or recurrent medullary thyroid carcinoma five or more years after total thyroidectomy. A longer period of evaluation will be necessary to confirm that they are cured.

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N Engl J Med 2005;353:1105-13.

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MEDULLARY THYROID CARCINOMA, a cancer of the parafollicular (C) cells, develops in virtually all patients with multiple endocrine neoplasia (MEN) type 2A (MEN-2A) or type 2B (MEN-2B) and in those with familial medullary thyroid carcinoma.¹⁻³ Medullary thyroid carcinoma is the most common cause of death in patients with these endocrinopathies, and total thyroidectomy that is performed before medullary thyroid carcinoma develops or spreads beyond the gland is currently the only curative treatment.

Over time, patients with MEN-2A, MEN-2B, or familial medullary thyroid carcinoma develop a spectrum of C-cell disorders, beginning with C-cell hyperplasia and progressing to intrathyroidal medullary thyroid carcinoma, regional lymph-node metastases, and distant metastases.⁴ C cells secrete the hormone calcitonin, and blood levels of this polypeptide serve as a sensitive tumor marker as well as an indicator of the C-cell mass.⁵ Intravenously administered calcium and pentagastrin are potent calcitonin secretagogues that markedly enhance the sensitivity of the calcitonin assay.⁶

In the early 1990s, it was shown that mutations in the *RET* proto-oncogene cause MEN-2A, MEN-2B, and familial medullary thyroid carcinoma.⁷⁻⁹ Thus, by direct DNA analysis, it is possible to identify persons within kindreds with these syndromes who have inherited a mutated *RET* allele and in whom medullary thyroid carcinoma is destined to develop. This discovery changed the treatment of patients at risk for hereditary medullary thyroid carcinoma, since it provided a rationale to perform thyroidectomy either before medullary thyroid carcinoma developed or while the cancer was confined to the gland.¹⁰

On the basis of clinical evaluation and biochemical testing, we sought to determine whether young patients with the MEN-2A genotype who had no physical evidence of medullary thyroid carcinoma were free of disease five or more years after total thyroidectomy. We also sought to determine whether the outcome of surgery is related to the specific *RET* codon mutation, the preoperative stimulated plasma calcitonin level, the age of the patient at the time of total thyroidectomy, the histologic status of the resected thyroid gland, the presence or absence of cervical lymph-node metastases, or all of these factors.

METHODS

SUBJECTS

In 1993, our clinic initiated a genetic screening program for patients at risk for MEN-2A, MEN-2B, and familial medullary thyroid carcinoma. We identified 85 patients who had inherited a mutated allele that is characteristic of MEN-2A. Each patient underwent total thyroidectomy, with the exception of two children (a 9-year-old child with cerebral palsy and a 12-year-old child with autism) whose parents did not consent to surgery.

Our study concerns the 50 patients consecutively identified as having MEN-2A who were 19 years of age or younger (range, 3 to 19 years; mean, 10 years) at the time of total thyroidectomy and who were evaluated for evidence of medullary thyroid carcinoma at least 5 years postoperatively (range, 5 to 10 years; mean, 7 years). Excluded from the analysis were 24 patients who were older than 19 years of age at the time of total thyroidectomy and 9 patients who, although younger than 19 years at the time of surgery, were evaluated only one to four years postoperatively.

The protocols and informed-consent documents for genetic testing, genetic counseling, total thyroidectomy, and postoperative evaluation were reviewed and approved by the institutional review boards at the Washington University School of Medicine and the Duke University School of Medicine. All patients and legal guardians of patients who were minors gave written informed consent to participate in the study.

CLINICAL AND HORMONAL ASSESSMENT

For each patient, a medical history was obtained and a physical examination performed. In addition, plasma calcitonin levels were determined before and after provocative testing with calcium and pentagastrin.⁶ Pentagastrin (Peptavlon) was obtained from Wyeth-Ayerst until 1999 and thereafter from Cambridge Laboratories. Five or more years after total thyroidectomy, all patients were evaluated by physical examination and by the determination of plasma calcitonin levels; 48 patients were tested both before and after the combined infusion of calcium and pentagastrin, 1 patient was tested with calcium gluconate alone, and 1 was tested with pentagastrin alone.

Plasma calcitonin levels were determined with the use of assay kits from the Nichols Institute (Quest Diagnostics). Until 1996, calcitonin levels were determined with the use of a radioimmunoassay; thereafter, these measurements (including studies performed five or more years after total thyroidectomy) were made by a chemiluminescent assay. In all patients, postoperative blood calcium levels were determined by atomic absorption analysis.

GENETIC TESTING

DNA was isolated from peripheral-blood leukocyte samples with the use of standard methods. Patients and at-risk family members were screened for mutations in the *RET* proto-oncogene with the use of polymerase chain reaction (PCR) amplification and direct sequencing for exons 10, 11, 13, and 14, which are known to harbor pathogenic MEN-associated mutations.¹¹⁻¹³ Each exon region was amplified with the use of two different pairs of PCR primers to avoid any possible amplification bias, and the DNA sequence from both strands was determined. PCR amplification of exon 16, coupled with restriction-enzyme digestion, was used to detect the codon 918 mutation associated with MEN-2B. Some point mutations in exons 10, 11, 13, and 14 also involve restriction sites, and for these, restriction-fragment-length polymorphism assays were substituted for PCR sequencing of DNA.^{9,11,13} We required concordance of the sequence data or restriction-fragment-length polymorphism patterns from both PCR products, both DNA strands, or all of these in order to report test findings.

OPERATIVE TECHNIQUE

One of four surgeons performed a total thyroidectomy on each of the 50 patients. Each surgeon followed an established operative protocol consisting of removal of the entire thyroid gland (including the posterior capsule) and resection of lymph nodes in the central zone of the neck (from the hyoid bone to the thoracic inlet and laterally to the carotid arteries). The parathyroid glands were removed and then placed as intramuscular autografts, either in the nondominant forearm (if the patient had a *RET* mutation associated with a high incidence of parathyroid hyperplasia) or in the neck.¹⁴

POSTOPERATIVE MANAGEMENT

Patients began taking oral calcium and vitamin D after total thyroidectomy and were discharged after the serum calcium level was documented to be

normal or near normal and after a calcium-pentagastrin stimulation test was performed. Thereafter, patients were evaluated by our group in collaboration with the patients' referring physicians. Our group performed annual physical examinations and intermittent calcitonin testing after thyroidectomy.

HISTOLOGIC ANALYSIS

The thyroid glands and soft tissues from the lymph-node dissection in the central zone were fixed in formalin and embedded in paraffin. Sections stained with hematoxylin and eosin were evaluated for the presence of medullary thyroid carcinoma according to the criteria of Wolfe et al.⁴ and Mendelsohn et al.¹⁵

STATISTICAL ANALYSIS

The marginal binomial proportions were estimated with the use of empirical proportions and exact Clopper-Pearson interval estimators.¹⁶ The discrepancy between two continuous distributions was assessed with the use of the exact Wilcoxon rank-sum test.¹⁷ The discrepancy between two binomial proportions was assessed with the use of Fisher's exact test for contingency tables.¹⁸ The association between clinical covariates and binomial responses was investigated with the use of conditional logistic regression.¹⁹ All P values and confidence intervals are two-sided and have not been adjusted to account for multiplicity.

The conditional logistic-regression analyses were carried out with the use of LogXact software (version 5, Cytel). All other statistical analyses were carried out with the use of R software (version 1.9.1, R Project for Statistical Computing). The implementation of the exact Wilcoxon rank-sum test¹⁷ found in the 0.8-8 version of the exactRankTests was used.

The study design, data gathering, data analysis, and writing of the manuscript were carried out by the study investigators.

RESULTS

DIRECT DNA ANALYSIS FOR *RET* MUTATIONS

RET mutations in the 50 patients with MEN-2A were found at codons 609 (3 patients), 611 (1 patient), 618 (11 patients), 620 (16 patients), and 634 (19 patients). Of the six patients who had evidence of persistent or recurrent medullary thyroid carcinoma after total thyroidectomy, two had mutations

in codon 618, 620, or 634. There was no statistical evidence that the presence of persistent or recurrent medullary thyroid carcinoma was dependent on the specific codon mutation ($P=0.92$).

PATHOLOGY

Thyroid Gland

On gross and histologic examination of the thyroid glands, 17 patients (median age, 6 years) had either no evident disease or isolated C-cell hyperplasia, whereas 33 patients (median age, 10 years) had microscopic or macroscopic evidence of medullary thyroid carcinoma. The age distribution of patients with either no evident disease or isolated C-cell hyperplasia differed from that of patients with medullary thyroid carcinoma ($P=0.02$).

Lymph Nodes

Lymph nodes from the central zone of the neck were resected in all but 1 of the 50 patients. The number of nodes removed ranged from 3 to 30 (mean, 10.9 nodes per patient). Metastases to regional nodes were found in three patients (one at 10 years of age, with 2 of 6 nodes positive, and two at 11 years of age, with 3 of 11 nodes positive and 1 of 8 nodes positive, respectively). In two of the three patients with metastases to regional nodes, plasma calcitonin levels became elevated during the postoperative period, indicating persistent or recurrent disease. The data suggest that patients with negative nodes had a lower incidence of persistent or recurrent medullary thyroid carcinoma than those with positive nodes ($P=0.04$).

PLASMA CALCITONIN LEVELS

Preoperative Levels

The age distribution of the 27 patients (median age, 11 years) with elevated preoperative plasma calcitonin levels after stimulation appeared to differ, if slightly, from that of the 23 patients (median age, 7 years) with normal preoperative levels ($P=0.05$).

Postoperative Levels

Forty-four of the 50 patients evaluated after total thyroidectomy had no evidence of persistent or recurrent disease, as evidenced by normal findings on a physical examination and by plasma calcitonin levels that were at or below the limits of detection of the assay before and after calcium and pentagastrin stimulation (proportion, 0.88; 95 percent confidence interval, 0.76 to 0.95) (Fig. 1A). In the remaining six patients, basal or stimulated

plasma calcitonin levels became elevated after total thyroidectomy, indicating persistent or recurrent medullary thyroid carcinoma. Patient 24 had basal plasma calcitonin values that exceeded the normal range in the immediate postoperative period and that continued to increase on subsequent testing (Fig. 1B and Tables 1 and 2). Stimulated plasma calcitonin values in this patient increased but remained within the normal range immediately after total thyroidectomy and rose above the normal range six years later.

Patient 13 had an elevated basal plasma calcitonin level three years after total thyroidectomy (Tables 1 and 2). The stimulated plasma calcitonin levels then increased gradually and reached levels above the normal range nine years after total thyroidectomy.

In Patients 1, 5, 19, and 36, basal plasma calcitonin levels were undetectable at all testing points after total thyroidectomy (Tables 1 and 2). However, the stimulated levels were elevated above basal levels but within the normal range. In Patient 1, the stimulated plasma calcitonin levels rose progressively but remained within the normal range five and eight years after total thyroidectomy. Nine years after surgery, Patient 19 had stimulated plasma calcitonin levels that had increased but that remained within the normal range. In Patient 5, stimulated plasma calcitonin levels had increased minimally above the basal level at five years. Patient 36 had undetectable stimulated plasma calcitonin levels three years after total thyroidectomy but a minimal increase above the basal level at six years.

TIMING OF SURGERY

Of 28 patients who were 8 years of age or older (median age, 13.5 years) at the time of total thyroidectomy, postoperative stimulated plasma calcitonin levels subsequently became elevated in 6. Postoperative stimulated levels remained undetectable in 22 patients who were 7 years of age or younger (median age, 5.5 years) at the time of total thyroidectomy ($P=0.03$). The specific cutoff age of eight years was chosen post hoc, and since the P value presented has not been adjusted accordingly, the observation should be interpreted with caution.

We attempted to perform a multivariate logistic analysis, with evidence of persistent or recurrent disease as the response and, as candidate covariates, patients' age at the time of thyroidectomy, the specific *RET* codon mutation, thyroid histologic

findings, preoperative calcitonin values, and regional lymph-node metastases. Because of the sparseness of the covariate structure, the resulting estimates were deemed to be unreliable and are not presented in this article.

PARATHYROID FUNCTION

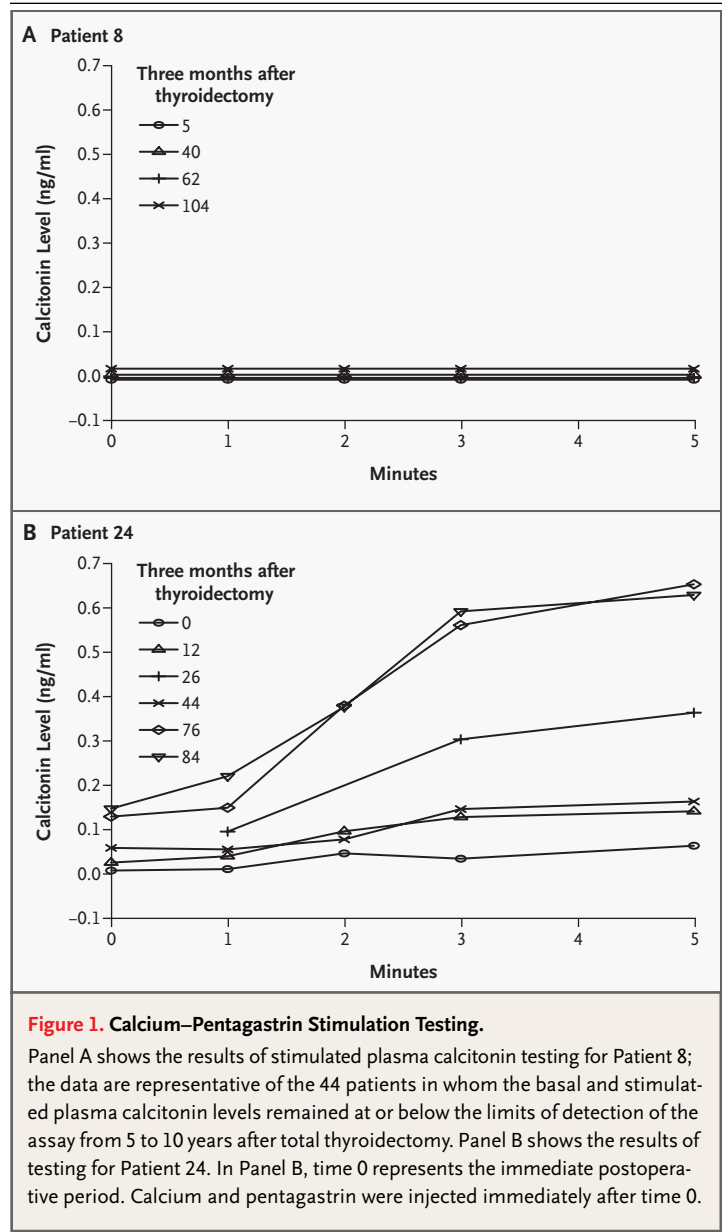
The postoperative serum calcium levels were evaluated at least one year postoperatively in all patients who underwent total thyroidectomy and dissection of the lymph nodes in the central neck zone. Serum calcium levels were normal in 47 of the 50 patients (94 percent); however, in 3 patients (ages four, four, and six years at the time of surgery), calcium and vitamin D supplements were required to maintain the serum calcium levels within or near the normal range.

DISCUSSION

With the availability of molecular techniques that identify persons in whom a specific solid-organ cancer is destined to develop, we have had the opportunity to test the hypothesis that such a cancer can be prevented or cured by preemptive removal of the organ, either before the cancer develops or while it is in situ. In order to confirm this hypothesis, several conditions must be met: there must be a reliable method of identifying patients in whom cancer in a specific organ will develop, the organ must be expendable or its function amenable to suitable replacement, removal of the organ must pose minimal risk to the patient, and there must be a sensitive method to determine whether the patient has been rendered free of disease and remains so after removal of the organ.

The medullary thyroid carcinoma associated with the type 2 MEN syndromes meets each of these criteria. Direct DNA analysis for mutations in the *RET* proto-oncogene can identify members of kindreds with MEN-2A, MEN-2B, or familial medullary thyroid carcinoma in whom thyroid cancer will develop. Thyroxine can replace the function of the removed gland. Elective total thyroidectomy is a standard operative procedure associated with minimal morbidity and virtually no mortality. The medullary thyroid carcinoma cells secrete calcitonin, which serves as an excellent tumor marker that indicates persistent or recurrent medullary thyroid carcinoma after total thyroidectomy.

In our study, we established strict criteria for determining whether young patients with MEN-2A



were free of disease after total thyroidectomy. Each of the 50 patients was evaluated by physical examination and calcitonin testing (with calcium and pentagastrin stimulation in all but 2 patients) five or more years after removal of the thyroid gland. A plasma calcitonin value above the normal basal level at a single time point after provocative testing was considered evidence of persistent or recurrent medullary thyroid carcinoma, even if the value fell within the range of plasma calcitonin values found in normal persons.

In 44 of the patients (88 percent), and in all of

those who underwent total thyroidectomy before eight years of age, there was no evidence of persistent or recurrent medullary thyroid carcinoma. Six patients who had increased basal or stimulated plasma calcitonin levels postoperatively were considered to have residual medullary thyroid carcinoma. Moderate elevations in plasma calcitonin levels have been reported in association with certain malignant tumors and benign inflammatory conditions, none of which were present in our patients.²⁰⁻²³ The progressive increases in stimulated plasma calcitonin levels after total thyroidectomy in patients with

medullary thyroid carcinoma are characteristic of an enlarging C-cell mass due to persistent or recurrent disease.

We evaluated the potential effects on outcome of several factors: age at the time of surgery, the histologic status of the resected thyroid gland (no evident disease or isolated C-cell hyperplasia vs. evidence of medullary thyroid carcinoma), the presence or absence of lymph-node metastases, the preoperative stimulated plasma calcitonin level, and a specific *RET* codon mutation. The data from our study suggest that there was a lower incidence of

Table 1. Characteristics of the Patients.*

Patient No.	Sex	Codon with <i>RET</i> Mutation	Age at Surgery yr	Pathological Findings	Lymph-Node Status <i>no. positive/ total no. resected</i>	Duration of Follow-up <i>mo</i>	Postoperative Calcitonin	
							Basal <i>pg/ml</i>	Peak
1†	F	618	19	MTC, MIC	0/5	97	<2	20
2	F	620	14	MTC, MAC	0/17	60	<3	<3
3	M	620	18	MTC, MIC	0/7	96	<4	<4
4	M	618	13	MTC, MAC	0/18	104	<3	<3
5†	M	620	14	MTC, MIC	0/30	60	<3	6
6	M	620	14	CCH	0/6	110	<3	<3
7	M	634	19	MTC, MIC	0/19	60	<2	<2
8	F	634	7	MTC, MIC	0/5	104	<3	<3
9	M	620	6	CCH	0/16	94	<4	<4
10	M	620	9	MTC, MIC	0/14	96	<4	<4
11	F	611	17	MTC, MIC	0/27	107	<3	<3
12	M	620	18	MTC, MAC	0/12	65	<2	<2
13†	M	634	16	MTC, MAC	0/6	109	89	1320
14	F	634	8	MTC, MIC	0/18	102	<3	<3
15	M	634	6	MTC, MIC	0/9	100	<2	<2
16	M	618	15	MTC, MAC	0/10	87	<4	<4
17	F	618	12	CCH	0/12	119	<3	<3
18	F	620	19	MTC, MIC	0/4	99	<3	<3
19†	F	634	8	MTC, MAC	0/4	109	<2	22
20	M	618	14	MTC, MIC	0/3	95	<3	<3
21	M	634	5	MTC, MIC	0/11	102	<2	<2
22	F	634	6	MTC, MAC	0/9	81	<4	<4
23	F	620	7	MTC, MAC	0/23	74	<4	<4
24†	M	620	10	MTC, MAC	2/6	84	130	653
25	F	609	18	CCH	0/22	121	<4	<4

persistent or recurrent disease in children who underwent total thyroidectomy before eight years of age and who had no metastases to cervical lymph nodes.

The most important consideration with regard to children known to have inherited a mutated *RET* allele, which is characteristic of MEN-2A, is the timing of the thyroidectomy. Currently, our practice is to perform total thyroidectomy when the child is between five and eight years of age, regardless of the *RET* codon mutation. In patients of this age, it is probably unnecessary to perform a central-zone

lymph-node dissection, since none of our patients younger than 11 years of age and none of the patients younger than 14 years described by Machens et al.²⁴ had lymph-node metastases at the time of total thyroidectomy. It should be noted, however, that Graham and colleagues reported lymph-node metastases in a six-year-old child with MEN-2A who underwent total thyroidectomy on the basis of increased stimulated plasma calcitonin levels.²⁵ Furthermore, one would expect the incidence of persistent hypocalcemia to be lower in children in whom central-zone lymph-node dissection is not

Table 1. (Continued.)

Patient No.	Sex	Codon with <i>RET</i> Mutation	Age at Surgery	Pathological Findings	Lymph-Node Status	Duration of Follow-up	Postoperative Calcitonin	
							Basal	Peak
			<i>yr</i>	<i>no. positive/total no. resected</i>		<i>mo</i>	<i>pg/ml</i>	
26	F	620	5	MTC, MIC	0/7	74	<4	<4
27	M	634	7	MTC, MIC	0/5	82	<2	<2
28	M	620	5	NED	0/9	84	<3	<3
29	M	634	9	MTC, MAC	0/4	96	<3	<3
30	F	634	7	MTC, MAC	0/11	101	<2	<2
31	M	618	9	MTC, MAC	0/5	79	<2	<2
32	M	620	9	CCH	0/6	73	<4	<4
33	M	620	3	CCH	0/20	73	<4	<4
34	F	620	7	CCH	0/9	73	<4	<4
35	M	618	16	MTC, MAC	0/20	81	<4	<4
36†	F	618	11	MTC, MAC	3/11	70	<3	7
37	F	609	13	NED	0/0	89	<2	<2
38	F	634	5	MTC, MAC	0/16	64	<4	<4
39	M	634	4	CCH	0/11	64	<4	<4
40	F	618	4	CCH	0/6	63	<4	<4
41	M	620	6	NED	0/9	77	<2	<2
42	F	634	6	CCH	0/13	78	<2	<2
43	M	634	5	CCH	0/7	73	<2	<2
44	F	634	11	MTC, MAC	1/8	72	<2	<2
45	F	618	5	CCH	0/11	73	<2	<2
46	M	618	7	CCH	0/14	73	<2	<2
47	M	634	12	MTC, MAC	0/17	66	<2	<2
48	F	634	5	MTC, MIC	0/9	60	<2	<2
49	M	634	5	MTC, MIC	0/3	60	<3	<3
50	F	609	13	NED	0/4	60	<3	<3

* MTC denotes medullary thyroid carcinoma, MIC microinvasive carcinoma (visible only microscopically), MAC macroinvasive carcinoma (grossly visible), CCH C-cell hyperplasia, and NED no evidence of disease.

† This patient had evidence of persistent or recurrent medullary thyroid carcinoma.

Table 2. Plasma Calcitonin Levels before and after Calcium–Pentagastrin Stimulation in Patients with Persistent or Recurrent Disease after Surgery.*

Patient No.	Age	Sex	Calcitonin Level (pg/ml)†											
			Immediate Postoperative Period		1 Year		2 Years		3 Years		5–7 Years		8–10 Years	
			basal	peak	basal	peak	basal	peak	basal	peak	basal	peak	basal	peak
24	10	M	9	65	26	142	ND	370	60	164	130	653	NT	NT
13	16	M	<4	<4	NT	NT	NT	NT	11	22	14	46	89	1320
1	19	F	<3	<3	NT	NT	NT	NT	<2	<2	<3	7	<2	20
19	8	F	<3	<3	NT	NT	NT	NT	<3	<3	<3	<3	<2	22
5	14	M	<3	<3	NT	NT	NT	NT	<3	<3	<3	6	NT	NT
36	11	F	<3	<3	NT	NT	<3	<3	<3	<3	<3	7	NT	NT

* ND denotes not done, NT not tested.

† Basal denotes plasma calcitonin levels before calcium–pentagastrin stimulation, and peak denotes plasma calcitonin levels after stimulation. Normal values are 4 pg per milliliter or less (basal) and 70 pg per milliliter (peak) in girls and 8 pg per milliliter or less (basal) and 491 pg per milliliter (peak) in boys.

performed at the time of total thyroidectomy than in those in whom it is performed.

Although the data from our study are encouraging, definitive guidelines for the operative treatment of children with MEN-2A, MEN-2B, and familial medullary thyroid carcinoma will require that

large numbers of patients be evaluated for a longer period postoperatively to confirm that prophylactic total thyroidectomy prevents or cures hereditary medullary thyroid carcinoma.

Supported by grants (PO1CA53524 and MO1RR00036) from the National Institutes of Health.

REFERENCES

- Steiner AL, Goodman AD, Powers SR. Study of a kindred with pheochromocytoma, medullary thyroid carcinoma, hyperparathyroidism and Cushing's disease: multiple endocrine neoplasia type 2. *Medicine (Baltimore)* 1968;17:371-408.
- Chong GC, Beahrs OH, Sizemore GW, Woolner LH. Medullary carcinoma of the thyroid gland. *Cancer* 1975;35:695-704.
- Famdon JR, Leight GS, Dilley WG, et al. Familial medullary thyroid carcinoma without associated endocrinopathies: a distinct clinical entity. *Br J Surg* 1986;73:278-81.
- Wolfe HJ, Melvin KEW, Cervi-Skinner SJ, et al. C-cell hyperplasia preceding medullary thyroid carcinoma. *N Engl J Med* 1973; 289:437-41.
- Pomares FJ, Rodriguez JM, Nicolas F, et al. Presurgical assessment of the tumor burden of familial medullary thyroid carcinoma by calcitonin testing. *J Am Coll Surg* 2002; 195:630-4.
- Wells SA Jr, Baylin SB, Linehan WM, Farrell RE, Cox EB, Cooper CW. Provocative agents and the diagnosis of medullary carcinoma of the thyroid gland. *Ann Surg* 1978; 188:139-41.
- Donis-Keller H, Dou S, Chi D, et al. Mutations in the RET proto-oncogene are associated with MEN-2A and FMTC. *Hum Mol Genet* 1993;2:851-6.
- Mulligan LM, Kwok JBH, Healey CS, et al. Germ-line mutations of the RET proto-oncogene in multiple endocrine neoplasia type 2A. *Nature* 1993;363:458-60.
- Carlson KM, Dou S, Chi D, et al. Single missense mutation in the tyrosine kinase catalytic domain of the RET protooncogene is associated with multiple endocrine neoplasia type 2B. *Proc Natl Acad Sci U S A* 1994; 91:1579-83.
- Wells SA Jr, Chi DD, Toshima K, et al. Predictive DNA testing and prophylactic thyroidectomy in patients at risk for multiple endocrine neoplasia type 2A. *Ann Surg* 1994; 220:237-50.
- Chi DD, Toshima K, Donis-Keller H, Wells SA Jr. Predictive testing for multiple endocrine neoplasia type 2A (MEN-2A) based on detection of mutations in the RET proto-oncogene. *Surgery* 1994;116:124-33.
- Bolino A, Schuffenecker I, Luo Y, et al. RET mutations in exons 13 and 14 of FMTC patients. *Oncogene* 1995;10:2415-9.
- Human Genome Mutation Database Cardiff. RET protooncogene. (Accessed August 22, 2005, at <http://archive.uwcm.ac.uk/uwcm/mg/search/120346.html>.)
- Mulligan LM, Eng C, Healey CS, et al. Specific mutations of the RET proto-oncogene are related to disease phenotype in MEN-2A and FMTC. *Nat Genet* 1994;6:70-4.
- Mendelsohn G, Eggleston JC, Weisburger WR, Gann DS, Baylin SB. Calcitonin and histaminase in C-cell hyperplasia and medullary thyroid carcinoma: a light microscopic and immunohistochemical study. *Am J Pathol* 1978;92:35-43.
- Clopper CJ, Pearson ES. The use of confidence or fiducial limits illustrated in the case of the binomial. *Biometrika* 1934; 26:404-13.
- Hajek J, Sidak Z, Sen PK. Theory of rank tests. 2nd ed. San Diego, Calif.: Academic Press, 1999:96-7.
- Cox DR, Snell EJ. Analysis of binary data. 2nd ed. New York: Chapman and Hall, 1989:48.
- McCullagh P, Nelder JA. Generalized linear models. 2nd ed. New York: Chapman and Hall, 1989:248-55.

20. Roos BA, Lindall AW, Baylin SB, et al. Plasma immunoreactive calcitonin in lung cancer. *Endocr Res Commun* 1979;6:169-90.
21. Whang KT, Steinwald PM, White JC, et al. Related serum calcitonin precursors in sepsis and systemic inflammation. *J Clin Endocrinol Metab* 1998;83:3296-301.
22. Milhaud G, Calmette C, Taboulet J, Julienne A, Moukhtar MS. Hypersecretion of calcitonin in neoplastic conditions. *Lancet* 1974;1:462-3.
23. di Sant'Agnese PA. Neuroendocrine differentiation in human prostatic carcinoma. *Hum Pathol* 1992;23:287-96.
24. Machens A, Niccoli-Sire P, Hoegel J, et al. Early malignant progression of hereditary medullary thyroid cancer. *N Engl J Med* 2003;349:1517-25.
25. Graham SM, Genel M, Touloukian RJ, Barwick KW, Gertner JM, Torony C. Provocative testing for occult medullary carcinoma of the thyroid: findings in seven children with multiple endocrine neoplasia type IIa. *J Pediatr Surg* 1987;22:501-3.

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