

C-Cell Hyperplasia and Medullary Thyroid Carcinoma: Clinicopathological and Genetic Correlations in 66 Consecutive Patients

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Routine calcitonin (CT) assay programs and genetic testing for *RET* proto-oncogene mutations have consistently modified the management and understanding of C-cell proliferative disorders. We report a series of 66 consecutive patients with C-cell hyperplasia (CCH) or medullary thyroid carcinoma (MTC) observed in our institution within an 8-year time period. All the patients had a preoperative basal CT assay and an *RET* proto-oncogene sequencing. Seventeen patients (F-M ratio: 8:9, mean age: 29.7 y) had a multiple endocrine neoplasia Type 2: 3 children <10 years of age had CCH only, and 14 patients had an MTC, with neoplastic CCH in 10/14 cases. Twenty-seven patients (F-M ratio: 18:9, mean age: 56.6 y) had a sporadic MTC, with physiological CCH in 8 and neoplastic CCH in 3 cases. Twenty-two men (mean age: 46.2 y) had CCH only (physiological CCH in 17 men and neoplastic CCH in 5). We conclude that (1) clinical and pathological characteristics (familial MTC, tumor multifocality, neoplastic CCH) usually associated with hereditary MTC may be misleading and that on the contrary, *RET* sequencing gives no false positive result; (2) sporadic neoplastic CCH accompanies (and probably precedes) a number of sporadic MTC; and (3) women presenting with a sporadic elevated basal CT have a 100% risk of having an MTC (15/15), but this risk is 3-fold less in men (31%), who will most often have CCH only (69%).

KEY WORDS: C-cell hyperplasia, Gender, Medullary thyroid carcinoma, Microcarcinoma, *RET*.

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Medullary thyroid carcinoma (MTC) is a rare neoplasm, which is hereditary in 25% of the cases, in multiple endocrine neoplasia Type 2 (MEN 2) families (1). During the past 10 years, two major tests have modified the current approach and presentation of MTC. First of all, the development of calcitonin (CT) screening programs in routine endocrinological practice has proven to be effective in the early detection of a number of MTCs (2–4). As a consequence, pathologists have been confronted with a dramatic increase in micro-MTC diagnosis ratio, as illustrated by recent series (5–7). On the other hand, this routine biological screening has also been responsible for the detection of an increasing number of patients presenting with C-cell hyperplasia (CCH) only (3, 8), in which thyroidectomy might be considered unjustified. Moreover, a highly sensitive and specific genetic test is now available to identify the germline mutations of the proto-oncogene *RET* in affected members of MEN 2 families. In MEN 2, MTC is characterized by a high penetrance (>90%; 9), and since 1997 there has been a consensus that the decision to perform a thyroidectomy should be based predominantly on the result of genetic testing (10). Therefore, affected children with a positive genetic testing are now often treated at a preneoplastic stage, called *neoplastic CCH* or *in situ MTC* (1, 11, 12).

In this study, we describe the clinicopathologic features of a series of MTC and CCH cases with complete biological and genetic testing, with emphasis on the practical consequences of these new diagnosis approaches in current diagnostic pathology and on the correlations between the various C-cell proliferative processes and clinical presentation.

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MATERIALS AND METHODS

Ninety-seven consecutive patients, excluding consultation cases, with an MTC or CCH diagnosis were retrieved in our pathology department files over a period of 8 years (from 1993 to 2000), representing 2.9% of 3342 thyroid pathological specimen examined during this time period. Thirty-one patients with nontotal thyroidectomy, with no or partial genetic testing, or with incomplete biological or clinical data were excluded.

Clinical, Biological and Genetic Studies

The medical records were reviewed for the following data: age, sex, medical complaint, preoperative levels of calcium, free thyroxin, thyroid stimulating hormone and thyroid auto-antibodies, associated thyroid pathology, and familial screening. Serum CT was measured by an immunometric assay using two antibodies (ElsahCT; Cis-Bio international, Gif sur Yvette, France), and basal CT values ≤ 10 pg/mL were considered normal, according to the French Calcitonin Tumors Study Group (GETC; 13). In patients who underwent a pentagastrin stimulation test, the response was expressed as the peak CT value after injection and was considered normal if this peak did not exceed 15 pg/mL in children <15 years of age and 30 pg/mL in adults (14). All the patients gave their consent to a germline mutation testing of the *RET* proto-oncogene. DNA was extracted from peripheral blood, then amplified by polymerase chain reaction. A negative result (no mutation) was considered after examination of exons 8, 10, 11, 13, 14, 15, and 16. The diagnosis and subclassification of MEN 2 was made according to the international consensus guidelines (9).

Pathological Study

All the specimens were submitted to the same routine pathological procedure that has been previously described (15) and were reviewed by two pathologists (SG, NJ) without knowledge of the clinical history and genetic status. Briefly, the thyroid gland was fixed in formalin, then horizontally sliced, and each section was embedded in paraffin. Serial 5- μ m sections of each block were used for routine stains (hematoxylin-eosin-saffron) and immunohistochemistry. Deparaffinized sections of all blocks were stained for CT (polyclonal rabbit anti-CT serum at 1/1000 dilution; DAKO SA, Paris, France). First of all, hematoxylin-eosin-saffron stained slides were screened to identify "neoplastic CCH," as proposed by Perry *et al.* (11). Neoplastic CCH was recognized by the presence of clusters of intrafollicular C-cells, easily identified on routine stains, made of mildly to moderately atypical cells

with nuclear pleomorphism, differing from adjacent follicular cells and resembling those identified in an MTC. In a second step, CT-stained slides were screened, and for each thyroid lobe the slide with the highest estimated C-cell density was submitted to semiquantitative evaluation: the number of low-power fields (LPF) at 100 \times magnification containing >50 C-cells (field area: 1.93 mm²) and the peak number of C-cells in one LPF were determined. A CCH diagnosis was made when at least three LPF containing >50 CT-immunostained cells were observed in one thyroid (16). According to Perry *et al.* (11), cases fulfilling the above-mentioned quantitative CCH criteria, but made of morphologically bland C-cells on routine slides, were diagnosed as "physiologic CCH." In a third step, we subclassified physiologic CCH according to the main growth pattern into focal, diffuse, and nodular forms, as previously proposed by Kaserer *et al.* (3, 7). Focal CCH corresponded to a segmental proliferation pattern of C-cells in the thyroid follicle, diffuse CCH was diagnosed when C-cells formed circumferential intrafollicular collars, and in nodular CCH, C-cells formed clusters completely obliterating follicular lumens. In the differential diagnosis between CCH (mainly nodular physiologic CCH and neoplastic CCH) and early micro-MTC, we considered the extension of C-cells through defects in the follicular basal lamina and the infiltration of the thyroid interstitium as malignancy criteria (17). Also, the existence of isolated infiltrating C-cells in a fibrous stroma and the identification of amyloid deposits were strong diagnostic criteria for micro-MTC. In a few cases, immunohistochemistry with anti-collagen Type IV antibody (monoclonal anti-collagen IV antibody at 1/150 dilution; DAKO SA, Paris, France) was applied to identify defects and/or reduplications of basal membrane, producing a microlobular or trabecular configuration in early MTC, as described by McDermott *et al.* (18). In contrast, we did not rely on cytological atypia for micro-MTC diagnosis, because it can be observed in neoplastic CCH (11).

Statistical Analysis

Statistical analysis was performed with Statview*SE+ Graphics (Abacus Concept, Inc., Chicago, IL). Either the Student's *t* test or, when necessary, a nonparametric test (Mann-Whitney) were applied to compare means, and the χ^2 test was used to compare frequency distributions. *P* values of $<.05$ were considered statistically significant.

RESULTS

Sixty-six patients (26 women, 40 men; sex ratio, F-M = 0.65), 5 to 79 years old (mean = 46.2 y) were

operated on in our institution between 1993 and 2000 with a consecutive CCH or MTC diagnosis. Forty-three patients sought consultation for a diffuse goiter or nodular thyroid disease, and 18 patients were potential MEN 2 carriers due to a prior familial MTC (17 cases) or to a concomitant bilateral pheochromocytoma (1 case). Five patients presented with an isolated hypercalcitoninemia (CT >10 ng/mL) identified after CT assay for a number of conditions such as a suspect cervical lymph node, diarrhea, elevated blood pressure, or vague recurrent symptoms of discomfort and flush. In most patients (59/66 [89.4%]), a C-cell disorder was suspected before surgery, because of either an elevated CT (41 patients), a suspected diagnosis of MEN 2 (4 patients), or both (14 patients). Consequently, a complete thyroidectomy was performed. Seven of 66 cases (10.6%) were fortuitous (normal CT level and no MEN 2 familial context) pathological discoveries on thyroidectomy specimen from patients operated on for another thyroid disease. In these cases, the thyroid glands were subsequently wholly included in paraffin for complete examination. The main pathological and clinical data are summarized in Table 1 and Table 2.

Clinicopathological Data According to Genetic Status

MEN 2 Carriers

Seventeen patients (8 women, 9 men) belonging to nine families were MEN 2 carriers with a documented *RET* mutation: 3 patients were members of MEN 2A families, whereas 14 patients belonged to fMTC (familial MTC) families. The *RET* mutation and MEN 2 subtype are shown in Table 3. Three patients were index cases presenting with an elevated CT and a nodular goiter (2 cases) or a bilateral pheochromocytoma (1 case). Pathological examination revealed multiple MTC associated with neoplastic CCH in two cases and without CCH in one. Fourteen patients were relatives of MEN 2 index cases. Seven patients were children <15 years of age (range, 5–12 y; mean, 7.9 y). Five of them had elevated basal CT levels, and two had only an abnormal pentagastrin test. Four children (6 to 12 y

old) already had one or several micro-MTCs, associated with neoplastic CCH in three cases and diffuse CCH in one. On the other hand, three children (5 to 10 y old) presented with CCH alone (diffuse CCH in 2 cases and focal CCH in 1 case). Seven patients were adults, 28 to 74 years old (mean: 42 y), with an elevated basal CT in four cases, an abnormal pentagastrin test only in two cases, and both a normal basal and stimulated CT in one case. All of them had multiple micro-MTCs with associated neoplastic CCH in five cases and no CCH in two cases.

Sporadic Cases

Forty-six patients (18 women, 28 men; sex ratio F-M: 0.64, mean age: 52.3 y) presenting with neither *RET* mutation nor family history of MEN 2 or MTC were included in this series.

Twenty-seven patients (18 women, 9 men; sex ratio F-M: 2) had a sporadic MTC (micro-MTC in 17 cases). The tumor was unique in 23 cases (including 12 micro-MTC), 3 patients had two MTC in the same thyroid lobe, and 1 patient had a bilateral MTC (two tumors in both lobes). In women, 17/18 tumors were unique, with no CCH (14 cases) or focal CCH (4 cases). In men, six patients had a unique MTC, with no CCH in two cases, diffuse CCH in two cases, nodular CCH in one case, and neoplastic CCH in one case. Three male patients had multiple micro-MTC, associated with diffuse CCH in one case and neoplastic CCH in two cases.

Nineteen men, aged from 28 to 66 years (mean: 46.2 y), had a sporadic CCH without MTC (sporadic CCH only). There were 3 focal, 7 diffuse, 4 nodular, and 5 neoplastic CCH. No woman had CCH only. Overall, CCH was present in 30/46 sporadic cases and was almost always bilateral (29/30 cases).

Undetermined Cases

In three patients, a thyroidectomy was performed on the basis of an abnormal basal CT discovered in a first-degree relative of an index MTC case. In each family, only the index case had an MTC (unique MTC in 2 patients, 2 micro-MTC in 1 case), and no *RET* mutation was identified in the seven above-mentioned exons in the family. The three patients

TABLE 1. Main Clinical and Biological Data in MEN 2 and Sporadic Cases

	Number	Age (mean)	Gender (F/M)	BasalCT (ng/mL) (median)	Stimulated CT (ng/mL) (median)
MEN 2	17	5–74 (29.7)	8/9	0–567 (13)	14–4794 (54.5), <i>n</i> = 14
MEN 2A	3	6–33 (15.3)	2/1	7–23 (14.5)	14–60 (37), <i>n</i> = 2
fMTC	14	5–74 (32.7)	6/8	0–567 (13)	18–4794 (61), <i>n</i> = 12
Sporadic C-cell disease	46	28–79 (52.3)	18/28	3–12350 (20.5)	29.6–2700 (121), <i>n</i> = 25
sMTC only	16	38–79 (56.0)	14/2	3–12350 (255)	108–2700 (245), <i>n</i> = 5
sMTC + CCH	11	46–72 (57.5)	4/7	3–1150 (21)	44–130 (116), <i>n</i> = 3
CCH only	19	28–66 (46.2)	0/19	8–51 (15)	29.6–188 (114), <i>n</i> = 17
Unclassified cases	3	36–60 (45)	0/3	17–53 (23)	41–85 (67), <i>n</i> = 3

BasalCT = basal calcitonin level; stimulated CT = peak calcitonin level after pentagastrin infusion test.

TABLE 2. Diagnosis According to Pathological Examination and RET Analysis in Patients Presenting in a Fortuitous Clinical Setting (Fortuitous Elevated Calcitonin Level or Fortuitous Pathological Examination Discovery) in Comparison with Patients Presenting in a Probable MEN 2 Context

Clinical Setting	Diagnosis			
	Sporadic MTC (+/-CCH)	Sporadic CCH only	MEN 2	CCH Only, Unclassified
Fortuitous (n = 48)				
Men (n = 29)	9	19	1	0
Women (19)	18	0	1	0
Probable MEN 2 (n = 18)				
Men (n = 11)	0	0	8	3
Women (n = 7)	0	0	7	0

NB = unclassified cases represent CCH cases in patient with a normal RET oncogene sequencing but with a previous MTC case in the family.

TABLE 3. RET Mutation Analysis Result in the Nine MEN 2 Families

Affected Codon and Amino-Acid Exchange	Exon	MEN 2 Subclassification
C634Y	11	MEN 2A
C634S	11	MEN 2A
C634S	11	MEN 2A
V804M F	14	fMTC
S891A	15	fMTC
S891A	15	fMTC
S891A	15	fMTC
V804 & R844L	14	fMTC
S891A	15	fMTC

were men aged from 36 to 60 y, with physiological CCH of various degrees (1 focal, 1 diffuse, and 1 nodular) with neither neoplastic CCH nor MTC.

Comparative Pathological Data

Sporadic versus Hereditary MTC

Forty-one patients had an MTC, among which 27 were sporadic MTC and 14 were MEN 2-associated MTC. The main clinicopathological differences between these two groups are summarized in Table 4. Patients with a hereditary MTC were significantly younger than patients with a sporadic MTC ($P = .0002$), but this difference was biased by the fact that 11/14 MEN 2 cases were identified by genetic screening. Only 3/14 patients were MEN 2 index cases, 33 to 69 years old (mean 51.7 y), with no significant age difference with sporadic cases. The presence of a previous MTC in the family was strongly associated with MEN 2 ($P = .0001$). In comparison with sporadic cases, hereditary MTC

were most often bilateral and multifocal ($P = .0001$ for both) and associated with CCH ($P = .005$). A neoplastic CCH was preferentially associated with hereditary MTC ($P = .0003$).

CCH Quantification and Classification

Forty-seven patients had CCH, which was morphologically classified as focal in 9 cases, diffuse in 14 cases, nodular in 6 cases, and neoplastic in 18 cases (Figs. 1–5). Quantitative data are summarized in Table 5. Among physiological CCH cases, there was a significant increase in C-cell density, estimated by the highest number of LPF containing >50 C-cells on one slide, between focal and diffuse CCH ($P = .033$), and between diffuse and nodular CCH ($P = .026$). There was also a tendency towards an increase in the peak C-cell number in one LPF from focal to diffuse and to nodular CCH, although it did not reach a statistical significance ($P = .13$ and $P = .08$, respectively). When estimated by the highest number of LPF containing >50 C-cells on one slide, there was no significant difference between physiological and neoplastic CCH. However, the latter contained a significantly higher peak C-cell number per LPF than physiological CCH as a whole ($P = .0007$). This difference was not significant between nodular CCH and neoplastic CCH. There was no statistical difference in preoperative CT level according to the different CCH subtypes, and there was no significant association between the presence of a chronic lymphocytic thyroiditis (6 cases) and the identification of a CCH.

TABLE 4. Comparison between Sporadic MTC and MEN 2-Associated MTC

	Age (mean)	Gender (F/M)	Bilaterality	Multifocality	Physiologic CCH			Neoplastic CCH
					Focal	Diffuse	Nodular	
MEN 2 associated MTC (n = 14)	5–74 (34.5)	8/9	11/14 (78.6%)	13/14 (92.9%)	1/14 (7.1%)			10/14 (71.4%)
Sporadic MTC (n = 27)	38–79 (56.6)	18/9	1/27 (3.7%)	4/27 (14.8%)	Focal	Diffuse	Nodular	3/27 (11.1%)
					0/14	1/14 (7.1%)	0/14	
					8/27 (29.6%)			
					Focal	Diffuse	Nodular	
					4/27 (14.8%)	3/27 (11.1%)	1/27 (3.7%)	

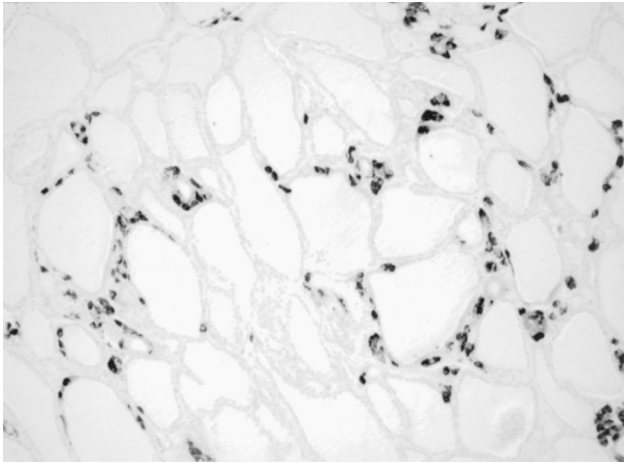


FIGURE 1. Sporadic focal C-cell hyperplasia. Thirty-three-year-old man, basal CT = 30 pg/mL, stimulated CT = 112 pg/mL. Immunoperoxidase reaction with anti-CT antibody; original magnification, 100 \times .

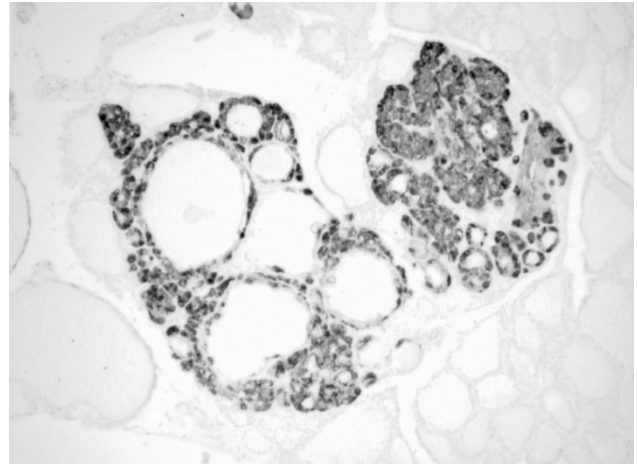


FIGURE 3. Sporadic C-cell hyperplasia, with diffuse (*left*) and nodular (*right*) pattern. Fifty-one-year-old man, basal CT = 18 pg/mL, stimulated CT = 156 pg/mL. Immunoperoxidase reaction with anti-CT antibody; original magnification, 100 \times .

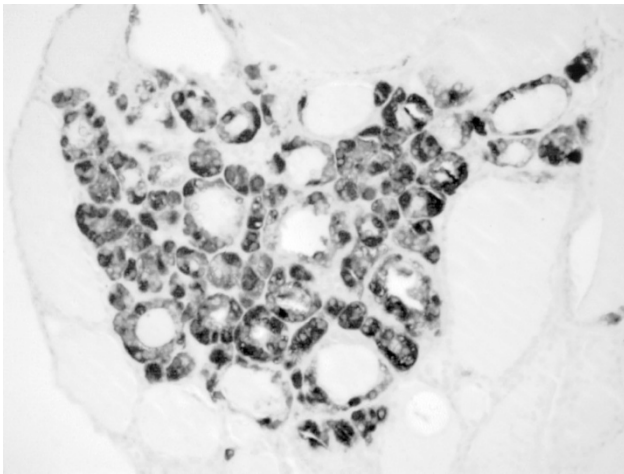


FIGURE 2. Sporadic diffuse C-cell hyperplasia. Forty-four-year-old man, basal CT = 29 pg/mL, stimulated CT = 145 pg/mL. Immunoperoxidase reaction with anti-CT antibody; original magnification, 200 \times .

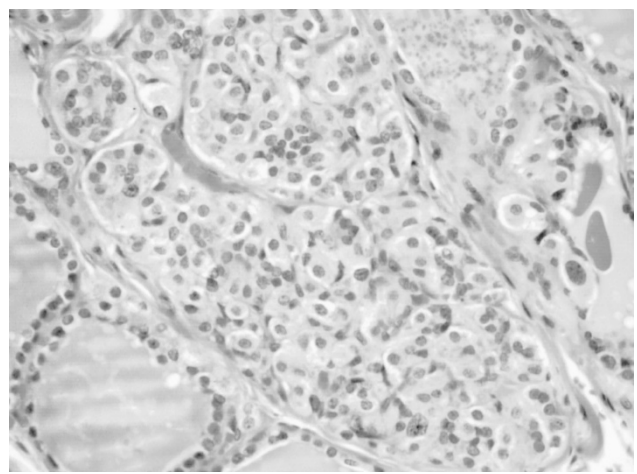


FIGURE 4. Sporadic neoplastic C-cell hyperplasia. Fifty-three-year-old man, basal CT = 14 pg/mL, stimulated CT = 129 pg/mL. Hematoxylin-eosin-saffron stain; original magnification, 400 \times .

DISCUSSION

We present a retrospective and longitudinal series of 66 patients with a CCH and/or MTC observed in our department between 1993 and 2000, representing about 3% of all the thyroid specimens examined during this time period. In our institution, CT assay is systematic in all patients presenting with a thyroid disease. This attitude has been justified in many reports (2–4), because it allows an earlier discovery of MTC, particularly at a micro-MTC stage, for which prognosis is far better than that for more advanced tumors. Nonetheless, it is worth noting that in 7/66 patients of our series, the C-cell disease (6 unique micro-MTC ranging from 0.5 to 6 mm in diameter, and 1 CCH only) was a fortuitous pathological discovery, because the preoperative CT was normal (range: 3–9 pg/mL, mean:

5.1 pg/mL)). Thus, clinicians and pathologists must be aware that a normal basal CT does not definitely exclude a micro-MTC.

A side effect of systematic CT assay was the concomitant increase in patients with a final diagnosis of sporadic CCH only, in which thyroidectomy might be considered unjustified. In our series, a sporadic CCH only (*i.e.*, CCH with neither familial MTC nor *RET* mutation) was observed in 19 male and in no female patients. This gender-associated CCH had a dramatic impact on the ratio of MTC in patients presenting with a fortuitous elevated basal CT (Table 2): all the women (15/15) and less than one third of the men (8/26) presenting in this setting had an MTC. In men presenting with a mildly elevated preoperative CT level (≤ 30 pg/mL), MTC was even rarer: 4/21 men with a micro-MTC (19%) and 17/21 men with CCH only (81%). It was not

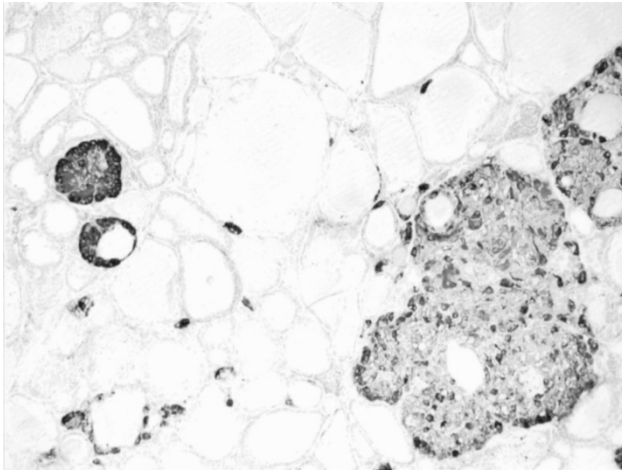


FIGURE 5. Medullary thyroid microcarcinoma (*right*), with adjacent neoplastic C-cell hyperplasia (*left*). Thirty-five-year-old man with multiple endocrine neoplasia Type 2 (fMTC), basal CT = 8 pg/mL, stimulated CT = 170 pg/mL. Immunoperoxidase reaction with anti-CT antibody; original magnification, 40 \times .

possible to distinguish between these two groups before surgery (mean basal CT of 21.5 and 18.5 pg/mL, respectively). Therefore, we consider that a total thyroidectomy is still currently recommended in men presenting with a fortuitous elevated CT. This gender-associated difference was not totally unexpected: in a previous quantitative image analysis study of 42 adult normal thyroid glands, we observed that C-cell density in men was more than twice that in women. Two women (15%) and 12 men (41%) had CCH (15). This difference was also observed in recent studies on patients operated on for an elevated CT (3, 8) and may be related to the usually higher CT plasma concentration observed in healthy men (19–21). Many observations point to a role of sex-steroid hormones on CT secretion and proliferation, although one poorly understood. Most studies have focused on the role of estradiol, and very few observations are available about progesterone and testosterone effects. Estrogen substitution has proven to stimulate CT secretion in postmenopausal women (22), and CT secretion *in vivo* and *in vitro* is also influenced by estrogens and by progesterone (23). Yang *et al.* (24) have shown that MTC cells in culture were mildly stimulated by estrogens and inhibited by tamoxifen, thus suggesting the expression of estrogen receptors (ER) in MTC

cells, but the expression of ER by normal human C-cells has not been demonstrated (25). This may suggest the role of other sex-steroid hormones and/or receptors: in this regard, Garcia-Ameijeiras *et al.* (26) have proposed that testosterone might influence CT secretion in man. As emphasized by Albores-Saavedra and Krueger (1), many more studies are needed to clarify the etiopathogenesis of physiologic (and particularly sex-associated) CCH.

Many investigators have reported on clinical and pathological characteristics of sporadic *versus* hereditary MTC (28–32). Our study confirms that the presence of another MTC in the family, as well as tumor multifocality and bilaterality, are strongly associated with MEN 2 (Table 4). However, as stated by Kaserer *et al.* (7), these criteria are unreliable in the individual patient to predict whether a familial risk is present or not. First of all, the presence of a previous familial MTC may be misleading: in our series, three patients had an elevated basal CT and a first-degree relative with an MTC. No *RET* mutation was identified in both the index cases and the patients, and pathological examination of total thyroidectomy specimen revealed CCH only. In these three patients, a familial MTC cannot be definitely ruled out in the absence of a complete *RET* gene sequencing, but our opinion is that they most probably represent sporadic CCH cases (>95% probability according to Brandi *et al.* (9)). This underlines the capacity of CT assay to identify non-genetically determined CCH cases (13, 33). We also observed a great frequency of tumor multicentricity and bilaterality in MEN 2 cases (92.9% and 78.6%, respectively) in comparison with sporadic cases (14.8% and 3.7%, respectively). Nonetheless, it is worth noting that on the basis of these criteria, 4/27 cases would have been erroneously considered as hereditary MTC, and 1/14 patients as a sporadic MTC.

For many years, the identification of a CCH in a thyroid containing an MTC has been considered an indicator of MEN 2 (30, 31). However, as we already observed (15), CCH is a rather common finding in adult thyroids, and lacks specificity as a MEN 2 predictor. In this series, we identified a CCH in 11/27 (41%) sporadic and in 11/14 (79%) MEN 2 cases. Perry *et al.* (11) have proposed morphological criteria to distinguish between physiological and

TABLE 5. C-Cell Quantification among 47 Patients with CCH

	Physiologic CCH (<i>n</i> = 29)			Neoplastic CCH (<i>n</i> = 18)
	Focal (<i>n</i> = 9)	Diffuse (<i>n</i> = 14)	Nodular (<i>n</i> = 6)	
Peak C-cell number per LPF*	70–250 (mean = 134)	100–340 (mean = 183)	170–360 (mean = 250)	130–390 (mean = 262.3)
Number of LPF containing more than 50 C-cells*	3–11 (mean = 6.0)	3–24 (mean = 11.4)	8–34 (mean = 20.0)	2–29 (mean = 11.4)

LPF = low power microscopic field ($\times 100$).

* In each thyroid lobe, quantification on the slide with the highest estimated C-cell density.

neoplastic CCH. The former requires immunohistochemistry and quantitative analysis to be identified with certainty. It has been reported with a high frequency ($\leq 69\%$) in normal adults (34) and in association with various conditions such as chronic lymphocytic thyroiditis, in the vicinity of follicular thyroid tumors, or in association with hypergastrinemia or hyperparathyroidism (1), and it has no documented malignant potential. The later is mainly recognized on cytological atypia, considered to reflect preneoplastic alterations associated with MEN 2. This is supported by a recent study by Diaz-Cano *et al.* (27), showing that MEN 2A-associated CCH had genetic and kinetic characteristics consistent with an intraepithelial neoplasia. Since 1996, a number of investigators have agreed that these two conditions actually represented different states in C-cell pathology (3, 7, 8, 12). Because the term "neoplastic CCH" seems contradictory in itself, several alternative denominations have been proposed, such as "*in situ*-MTC" (12, 28, 29), "thyroid intraepithelial neoplasia of C-cells" (7), or "C-cell intraepithelial neoplasia" (CCIN). We agree with this opinion but employed the term *neoplastic CCH* in this study because it is currently the most widely used.

The results of C-cell quantification in our series are summarized in Table 5. There was an increase in C-cell density as CCH progressed from focal to diffuse and from diffuse to nodular subtypes. On the other hand, there was no significant difference between C-cell density in neoplastic CCH and nodular physiologic CCH. Moreover, preoperative CT levels did not correlate with CCH subtypes. These data confirm that it is inappropriate, either from a practical or from a theoretical point of view, to try to distinguish between physiological and neoplastic CCH on quantitative grounds. In the first report by Perry *et al.* (11), the investigators identified neoplastic CCH in MEN 2 patients only. However, Scheuba *et al.* (8) have identified neoplastic CCH in 15/39 sporadic CCH cases, and a recent study by Kaserer *et al.* (7) reported the presence of neoplastic CCH in association with sporadic micro-MTC in 6/34 cases, suggesting that neoplastic CCH could also be the precursor of some sporadic MTC. We found neoplastic CCH in 10/14 (71.4%) MEN 2 cases but also in 8/46 (17.4%) sporadic cases. When associated with sporadic MTC (3/27 cases, 11.1%), neoplastic CCH was found close to infiltrative MTC, displaying the same kind of cytological atypia, a fact that may be an argument for a lineage between both lesions. As suggested by Kaserer *et al.*, we think that the first reports on the absence of CCH associated with sporadic MTC might have been biased by the important size of tumors, most of the thyroid parenchyma being replaced by tumor growth (3). As CT assays allow the earlier discovery

of sporadic MTC, often at a micro-MTC stage, it could soon become evident that sporadic MTC may also develop from neoplastic CCH, at least in a certain number of cases. On the other hand, it is worth noting that we found no neoplastic CCH in the vicinity of sporadic MTC in women (18 cases). Thus, alternative tumorigenesis pathways might also exist that do not necessitate a neoplastic CCH stage.

In conclusion, our study confirms that clinical and pathological characteristics often associated with hereditary MTC are currently overtaken by *RET* gene sequencing in most instances, as this test does not lead to false-positive conclusions. *RET* gene analysis should be proposed to each patient presenting with an MTC, and probably with a neoplastic CCH, for which we prefer the term *C-cell intraepithelial neoplasia*. This condition was mostly observed in hereditary MTC, but we also identified it in a number of sporadic cases. In this regard, we postulate that neoplastic CCH might be the precursor of a certain number of sporadic MTC. Moreover, our study reveals that in patients presenting with a fortuitous mild hypercalcitoninemia (*i.e.*, CT ≤ 30 pg/mL), the MTC risk is fivefold lesser in men (19%) than in women (100%). This emphasizes the great male prevalence of CCH only, which requires further investigations to be identified before an unjustified thyroidectomy.

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