Thyroid C-Cell Hyperplasia and Micronodules in Close Relatives of MEN-2 A Patients: Pitfalls in Early Diagnosis and Reevaluation of Criteria for Surgery

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Authors
Thyroid C-Cell Hyperplasia and Micronodules in Close Relatives of MEN-2A Patients: Pitfalls in Early Diagnosis and Reevaluation of Criteria for Surgery


In a large family with multiple endocrine neoplasia type 2A (MEN-2A), 20 patients were identified by the diagnosis of medullary thyroid carcinoma (MTC) and/or pheochromocytomas. Another five subjects had neck surgery on the basis of slightly increased results of a C-cell provocative test. Retrospectively, however, although the immunohistochemical diagnosis of C-cell hyperplasia was confirmed, the diagnosis of MEN-2 was doubtful in these five subjects, and the C-cell hyperplasia observed was probably within the limits of normal variation. The occurrence of C-cell hyperplasia in a normal population was investigated by a C-cell provocative test, as well as in random postmortem material. An increased number of C-cells in normal subjects is fairly common and may represent a physiologic condition rather than a neoplastic phenomenon. Identification of early expression of MEN-2 is difficult on the basis of slightly increased C-cell-provocative test results. To avoid total thyroidectomy in normal subjects, it seems reasonable to postpone surgery until the peak stimulated level of pure extractable calcitonin increases to more than 1,000 pg/mL. (Henry Ford Hosp Med J 1987;35:133-8)

Multiple endocrine neoplasia type 2A (MEN-2A) is a syndrome characterized by the concomitance of multiple pheochromocytomas and medullary thyroid carcinoma (MTC) combined with hyperparathyroidism. MTC is a malignant tumor of the thyroid originating in the C-cell system and occurs either in a sporadic or hereditary form. In hereditary cases the disease is inherited as an autosomal dominant trait, and tumor formation is invariably bilateral and multicentric (1). Calcitonin (CT) is a specific and sensitive marker of MTC (2). The short calcium (Ca) or pentagastrin provocative test allows early detection of patients with C-cell hyperplasia as the precursor of MTC (3,4).

Wolfe and DeLellis (5) studied thyroid sections from patients at risk for MTC and normal autopsy controls by immunohistochemical techniques. They established a close correlation between C-cell count and tissue CT content. They found up to ten C-cells per 10X field (ie, 100 C-cells per µL or 0.5 C-cells per mm²) in normal thyroids, and concluded that multiple, large clusters of C-cells must represent a pathological condition. Williams (6,7) considered nodular hyperplasia as the characteristic finding in the endocrine glands of patients with the MEN-2 syndromes. However, in a survey of a normal population, Gibson et al (8-10) and O'Toole et al (11) have shown that C-cell hyperplasia and/or micronodules are fairly common (5% to 10%), especially in children under age six and in the elderly. Since C-cell nodules and/or diffuse hyperplasia apparently occur commonly although MTC does not, these lesions probably possess only a low potential for malignancy, representing most likely a physiologic rather than a neoplastic phenomenon. If normal relatives of MEN-2 patients had this kind of C-cell hyperplasia, discrimination between C-cell hyperplasia as a preneoplastic precursor of MTC or as a physiologic phenomenon would be difficult.

Identification of large MEN-2 families in The Netherlands and our experience with periodic screening programs and central registration were reported earlier (12-15). As do most authors, we recommend early surgical removal of the entire thyroid gland because of the risk of malignant spread. Between 1975 and 1985, 25 family members underwent total thyroidectomy and central cervical lymph node dissection for suspected MTC. Five of the 25 patients had only C-cell hyperplasia demonstrated by the histopathologic findings. These five patients have been followed for four to nine years, by physical examination and laboratory tests at six-month intervals.

In the present study we reevaluated the results of the histopathologic findings and the postoperative course in all members of the kindred who underwent thyroidectomy. To assess the occurrence of C-cell hyperplasia in a normal population, we investigated basal and peak serum CT levels after stimulation in 100 normal subjects. We also analyzed 30 thyroid glands obtained at

Submitted for publication: March 9, 1987.
Accepted for publication: April 29, 1987.
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random from postmortem material and compared the distribution and amount of C-cells in these normal subjects with C-cell hyperplasia in our MEN-2A family members.

The purpose of this study was to define new criteria for early thyroid surgery in the offspring of MEN-2A patients and to discriminate between a physiologically hyperfunctional and hyperplastic C-cell system and the neoplastic state.

**Patients and Methods**

**Members of a MEN-2A kindred**

In this family (12), 165 members were examined (Fig 1). The penetrance of the syndrome is probably almost 100%, i.e., nearly all those individuals carrying the gene responsible for the disease will eventually show phenotypic expression of the syndrome. A member of the kindred was considered "normal" if both parents have no phenotypic signs of the syndrome and if the test results remained normal after age 20.

**Normal subjects**

One hundred normal individuals (males aged eight to 62 and females aged six to 61) underwent a short Ca infusion test. They had no evidence of abnormalities of Ca metabolism or bone disease as demonstrated by medical history, physical examination, and normal blood Ca. The investigation met the World Health Organization standards for human research.

**C-cell provocative test**

The overnight fasting subject was recumbent throughout the short Ca test (12). After blood sampling for basal CT level, an intravenous dose of 2.5 mg/kg of Ca was injected slowly in 30 seconds. Blood samples for CT were taken two and five minutes after the injection. According to the original criteria (12), total thyroidectomy was performed if the peak level of pure extractable human CT was more than three times the basal level or higher than 300 pg/mL pure extractable CT.

**CT radioimmunoassay**

CT radioimmunoassay (RIA) was carried out using synthetic human CT (Cibacalcine®, Ciba-Geigy Corp, Basel, Switzerland) as standard. 125I-CT as tracer, and goat-anti-synthetic human CT antiserum. This antiserum had no cross-reaction with the 1-10 amino acid (AA) portion of the human CT molecule but 100% cross-reaction with the 11-32 AA region. Separation of free and bound fractions was carried out with a charcoal-dextran suspension. The sensitivity was 20 pg/mL. Most normal subjects had values of up to 130 pg/mL. The coefficient of variation of the intraassay comparison was 5.5% and interassay was 3.2% at 100 pg/mL. The sensitivity could be raised to 2 pg/mL by immunoextraction with Reacti-gel (6x)® (Pearce Chemical Company, Rockford, IL) coupled antibodies.

**Tissue preparation techniques**

To study the degree of C-cell hyperplasia found in the affected members of our MEN-2A kindred, we compared the distribution and amount of C-cells in these cases with those from normal subjects. We investigated 30 thyroid specimens from older subjects obtained randomly from autopsy material. Most of these
subjects were male, and 25 were over 60 years old. They had had no known thyroid disease nor tumor metastases and the thyroid glands were grossly normal.

One midline sagittal slice of the lateral thyroid lobe was taken from each autopsy specimen. For immunocytochemical detection of CT in C-cells, an indirect horseradish peroxidase (HRP) staining technique was used. The tissue sections were incubated in the first step with rabbit-anti-CT antibodies, in the second step with swine-anti-rabbit immunoglobulins (DAKO), and in the third step with HRP and rabbit-anti-HRP antibodies (DAKO-PAP). The resulting enzyme-labeled complexes were visualized with the 3,3 diaminobenzidine tetrahydrochloride reaction (DAB). The nuclei were counterstained with hematoxylin.

We performed C-cell area measurements with a Videoplan III Kontron MOP apparatus (11). C-cells per mm² were counted. For comparison with the results of Gibson et al (8-10) who calculated the number of C-cells per µL, we assumed a section thickness of 5µm and multiplied our count by 200. Our histopathological criteria for C-cell hyperplasia were the presence of large amounts of diffusely scattered C-cells characterized by an increase in size and number of C-cells per follicle compared with normal controls (more than seven C-cells per follicle and cluster formation). In an early stage the follicular architecture is preserved. If proliferation of the C-cell system progresses, the C-cells may completely encircle, compress, and displace the follicular epithelium, and ultimately form solid intrafollicular aggregates of C-cells (nodules) with complete replacement of the epithelium and colloid. In benign C-cell hyperplasia, however, the follicular basement membrane is never disrupted, and there is no invasion of the interstitium.

Results

In this MEN-2A kindred (Fig 1), 165 members were examined. Ten patients underwent bilateral adrenalectomy for pheochromocytomas. Twenty-five subjects showed abnormal Ca-stimulation test results and underwent thyroidectomy and central cervical lymph node dissection (Fig 2). Histopathologic examination showed MTC in 20 patients and C-cell hyperplasia only in five patients. In these cases the entire thyroid specimens were carefully sliced, immunostained, and examined for CT reactivity. In ten of the 20 operated patients with MTC, peak CT levels remained elevated after surgery, which indicated the presence of metastases. Increase of peak CT levels was studied in close relatives of MEN-2 patients during consecutive years. Two types of increase were observed: 1) an increment or stimulation of 300 to 1,000 pg/mL (peak level may fluctuate even in consecutive tests, but in general the increase does not exceed 1,000 pg/mL), and 2) a progressive increase in consecutive tests to a level where the existence of MTC is obvious (Fig 3).

At pathology, two of the five patients with C-cell hyperplasia (18 and 19 years old) had nodules containing 94 and 100 C-cells, respectively. All five patients had normal peak CT levels after surgery (Figs 2 and 5). After several years follow-up, it now seems probable that at least three (D-3, D11-1, and D11-2) of these five patients do not carry the MEN-2 gene. In the cases of D11-1 and D11-2, their mother (aged 70 years) has never had any expression of the syndrome. Their brother and sister (D11-3

<table>
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<th>Pedigree number</th>
<th>Patients (Fig 1)</th>
<th>Highest C-Cell containing nuclei</th>
<th>Clusters containing nuclei</th>
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<tbody>
<tr>
<td>D11-1</td>
<td>18/M</td>
<td>4.72</td>
<td>30</td>
</tr>
<tr>
<td>D11-2</td>
<td>19/M</td>
<td>6.20</td>
<td>20</td>
</tr>
<tr>
<td>D10</td>
<td>55/M</td>
<td>2.13</td>
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<td>D3</td>
<td>59/M</td>
<td>2.76</td>
<td>10</td>
</tr>
<tr>
<td>D5</td>
<td>59/M</td>
<td>3.08</td>
<td>20</td>
</tr>
</tbody>
</table>
| ND = not detected.
and D11-4) show fluctuations in peak levels (D11-4 into a suspected level). Patients D3, D5, and D10 developed abnormal test results at about 60 years of age, and all their offspring (in case D3, nine children aged 24 to 44 years) have had normal test results.

**Peak increases in CT in the short Ca test in 100 normal subjects**

The CT responses were generally higher in men than in women in all decades (p < 0.001) (Fig 6). A negative correlation was noted between age and CT response (regression coefficient \( a = -0.096 \, \text{pg/mL/year}, r = -0.455; p < 0.03; \) this regression was more obvious for women than for men). Ten percent of men and 2% of women had an increase of more than 200 pg/mL.

C-cell counts in autopsy specimens ranged from less than 0.01 to 2.20 cells per mm². Four of 30 autopsy specimens showed C-cell hyperplasia morphologically similar to that of our five thyroidectomized kindred members. Two of these four specimens (from patients aged 87 and 77 years) showed nodules containing up to 100 and 236 C-cells, respectively, and clusters of 20 to 30 cells (Fig 7).

**Discussion**

In this MEN-2A family, five patients underwent surgery on the basis of slightly increased provocative test results suggesting C-cell hyperplasia (12,13). The diagnosis was confirmed by the pathology.

After several years, it is now questionable whether or not those five subjects really were carriers of the MEN-2 gene. Neither pheochromocytomas nor parathyroid gland hyperplasia has occurred. All 16 children of D3, D5, and D10 (aged 24 to 44 years) showed normal test results. Four of 30 autopsy specimens showed C-cell hyperplasia morphologically similar to that of our five thyroidectomized kindred members. Two of these four specimens (from patients aged 87 and 77 years) showed nodules containing up to 100 and 236 C-cells, respectively, and clusters of 20 to 30 cells (Fig 7).

**Fig 5 (left)—Increase in CT levels (6 CT) in consecutive years after Ca stimulation.** The four panels show different D family members (Fig 1). The mother, D11, who is a member of the MEN-2A kindred, has had normal test results since the start of the screening in 1975. Her eldest sons (D11-1 and D11-2) underwent surgery and had C-cell hyperplasia. Her youngest children (D11-3 and D11-4) were also suspected of having C-cell hyperplasia; they did not have surgery but are being followed carefully. Note that increments in CT fluctuate over successive years, but do not exceed 1,000 pg/mL. Also note that the father of these children (D11A), who is not a member of the kindred, also showed an increment of CT on stimulation. Panel 3 shows three other members (D3, D5, D10) of the family D in whom the diagnosis of C-cell hyperplasia was histologically confirmed. Patient D3 died from a myocardial infarction, and D5 died from a carcinoma of the lung.
years. Fig 1) have had normal CT test results. The mother of cases Dll-1 and Dll-2 has never shown phenotypical expression of the syndrome. Pathological examination of the thyroid from these individuals revealed intact follicular basement membranes and no follicular replacement by C-cell proliferation. The appearance of the nodules was similar to the benign C-cell hyperplasia often seen in neonates and young children. In retrospect, it is our opinion that at least three of these five cases were normal nonaffected family members.

In our study and that of others of normal individuals, CT response to stimulation is influenced by age and sex (16-25). In both sexes a progressive decrease in CT response to Ca stimulation was noted with age. CT was generally higher in men than in women in each age group. In about 10% of men and 2% of women the increase was higher than generally expected (greater than 200 pg/mL).

In a recent study of the effect of age on the number of CT-immunoreactive cells in the thyroid gland, O'Toole et al (11) found an extreme variability. Although an increase in the number of CT-immunoreactive cells with age was suggested, the values were not statistically significant because of the large standard deviation. Our results agree with theirs, and the C-cell densities in our five family members thyroidectomized for C-cell hyperplasia fell within this range of normal variation. Gibson et al (10) found that five of six patients with C-cell nodules in a series of 30 autopsy cases were older than 50 years, suggesting that C-cell clusters may be fairly common in a normocalcemic older population. Their earlier studies on normal children and young adults reported an extreme variability in the number of CT-immunoreactive cells (8). In 22 autopsies of accident victims younger than 30 years, they found three persons aged 22 to 29

![Fig 6](image)

**Fig 6**—Peak increases in CT in the short Ca test in 100 normal subjects (men, upper panel; women, lower panel). The increment in CT is greater in men than in women, and a progressive decrease with age can be noted.

![Fig 7](image)

**Fig 7**—Nodular hyperplasia of C-cells in two postmortem thyroid specimens (from patients aged 77 [left] and 87 years [right]). The basement membrane is left intact, and there is a clearly follicular arrangement. The cytoplasm of the C-cells is vacuolated.
with a far higher C-cell population density than the others (up to 120 cells per 10X field, ie, 1,200 cells per µL). It is also our opinion that C-cell clusters are fairly common in members of a normal population. C-cells may be distributed in diffuse hyperplasia or in nodules. No disruption or degeneration of follicular basement membrane was apparent in these reports of normal subjects. In general, the morphologic features resembled those observed in pediatric cases. These lesions probably have low malignant potential and may represent a physiologic rather than a neoplastic phenomenon. However, Livolsi and Feind (26) reported two cases of sporadic MTC originating in C-cell hyperplasia probably secondary to primary hyperparathyroidism.

In conclusion, on the basis of borderline increased CT levels in provocative tests, it is not possible to differentiate diffuse and/or nodular C-cell hyperplasia occurring as a variation within a normal population from a neoplastic state which precedes MTC. If patients are treated surgically on the basis of an increased CT level after provocation of 500 to 1,000 pg/mL, some will be found to have diffuse or nodular C-cell hyperplasia as a variation of a normal condition. To avoid unnecessary surgery and imposing the stigma of hereditary MEN-2 on a normal member of a kindred, one must be cautious in the interpretation of CT stimulation procedure results.

No recurrence of the disease after thyroidectomy was noted in our patients if the preoperative increment of CT was less than 10,000 pg/mL. On the basis of the results of this study, therefore, it seems justified to postpone thyroid surgery until a CT increase of 1,000 pg/mL occurs after provocation.

An increase in peak CT levels in consecutive years may support the diagnosis of preneoplastic C-cell population. It is important to standardize the provocative CT test protocol to insure reproducibility. If the injection of calcium or pentagastrin is given in less than 30 seconds or in a higher dose, a higher CT response is likely. For this reason the choice of provocative agents is less important than the careful performance of the test.

In the future, restriction fragment length polymorphism linkage studies (27,28) will provide insight into the location and types of derangement underlying the MEN-2 syndrome. Cloning of the mutant gene itself will make family screening for the MEN-2 syndrome much easier.

Acknowledgments

We are indebted to Drs. J. Seelen and M. Kroon (Westeinde Hospital, The Hague), Professor J.H.H. Thijssen (University Hospital Utrecht), and their laboratory staff for determination of catecholamines, metabolites, and CT levels. Thanks are also due to the nursing staff of the outpatient clinics and to J.M. Jansen-Schillhorn van Veen and I.G.J. Janssen for outstanding technical assistance and to E. den Aantrekker for the excellent elaborating of the manuscript.

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