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High-Sensitivity Serum Calcitonin Assays Applied to Screening for Thyroid C-Cell Disease in Multiple Endocrine Neoplasia Type 2A*

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Two serum calcitonin assays with sensitivities ≤ 10 pg/mL were compared to our standard radioimmunoassay (sensitivity 100 pg/mL) in multiple endocrine neoplasia type 2A (MEN 2A) screening. Values from the Nichols displacement radioimmunoassay averaged 38% higher than values from the CIS immunoradiometric assay; values from both were highly correlated, r = 0.845. In three individuals, both of the newer assays revealed abnormalities in pentagastrin tests three to four years before abnormalities were detected by the standard assay. Pentagastrin tests after total thyroidectomy were assayed by the newer methods in patients with medullary thyroid carcinoma (MTC) diagnosed at initial testing (group I); in patients with early MTC diagnosed by prospective screening (group II); and in patients with pure C-cell hyperplasia detected by prospective screening (group III). At least 64% of group I, at least 25% of group II, but none of group III had detectable postoperative C-cell function. Conclusions: 1) The previous estimate of 12 years as median age of onset of C-cell disease in MEN 2A is probably three to four years too old. 2) Patients diagnosed with early MTC by screening had not necessarily skipped a preneoplastic phase of C-cell hyperplasia. At least some early disease was not detected by the standard assay. Higher sensitivity assays should improve screening for C-cell disease by earlier disease detection. 3) Biochemical cure by thyroidectomy after the development of MTC is not as frequent as previously thought, but the apparent cure rate of pure C-cell hyperplasia remains 100%. (Henry Ford Hosp Med J 1992;40:227-31)

A large kindred with multiple endocrine neoplasia type 2A (MEN 2A), the J-kindred, has been the subject of a long-term study begun in 1969 to identify individuals with C-cell disease and medullary thyroid carcinoma (MTC) in the preinvasive stage. The disease is inherited in autosomal dominant fashion with a theoretical probability that each child of a gene carrier has a 50:50 chance of developing MTC. During the first two years of the study, 12 family members were found to have abnormal plasma calcitonin (CT) responses to calcium infusion as measured by radioimmunoassay (1,2); each was operated upon and found to have MTC (3). A prospective study of individuals at risk was then initiated in which stimulated CT secretion was determined at one- to two-year intervals. CT-secretion screening tests, with calcium as the secretagogue during the first few years and pentagastrin more recently, have identified many patients who have only C-cell hyperplasia or microscopic foci of carcinoma in a gland with C-cell hyperplasia and who have no evidence of metastatic disease (4-7). Similar studies have been reported by other groups (8-10). By pooling test results from the J-kindred with a large number of other kindreds, it has also been possible to calculate the probability of developing C-cell disease as a function of age (11,12). Our screening protocol is started at 5 years of age for children at risk. C-cell abnormalities are assessed by annual CT measurements before and after pentagastrin stimulation; surgery is advised after two successive abnormal tests. Almost all family members so advised have had thyroidectomy within a few months.

Although the majority of individuals with C-cell disease have been identified by prospective screening while in the premetastatic stage, this program has not identified every patient in the preneoplastic stage. Of the individuals found to have developed C-cell disease during prospective screening between 1972 and 1989, 11 of 24 had intrathyroidal foci of medullary carcinoma at the time of surgery (6). This finding suggests that the premalignant phase of C-cell hyperplasia (7) is absent or lasts less than two years in some affected persons, or, alternatively, that the screening test might be too insensitive to detect the earliest stages of C-cell hyperplasia. Another uncertainty is that the likelihood of cure by total thyroidectomy and central node dissection, the standard surgical procedure for C-cell disease (13,14), is not known for those patients treated after they develop local-

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ized foci of medullary carcinoma. Since most patients survive for many decades with known residual MTC, CT measurements must be used as surrogates for mortality data.

The CT radioimmunoassay (1,2) used since the inception of the J-kindred screening program has a sensitivity of 100 to 200 pg/mL. Two additional CT assays have become available that have sensitivity limits of about 10 pg/mL. It was anticipated that the use of more sensitive assays might permit the detection of affected individuals with C-cell disease at an earlier stage. Since 1986, results from these newer assays have been compared with results obtained with the standard assay in screening the J-kindred and another MEN 2A family, the C-kindred. By the use of the new CT assays in the prospective screening program and from measurements of CT in archived frozen serum samples from the J-kindred, evidence has emerged that the age of onset of C-cell hyperplasia may be earlier than previously thought, and that the cure rate in patients with minute foci of MTC may not be as high as our earlier findings indicated. These findings have important implications about the biology of MEN 2A and must be taken into account when calculating risks of genetic susceptibility when used in conjunction with analysis of polymorphic gene markers (15-18).

Patients and Methods

The J-kindred has been described in detail (3-7). The C-kindred, another MEN 2A family, is of French origin with family members living in France, the Quebec province of Canada, and Massachusetts with MTC occurring in three generations. Two affected individuals also have had pheochromocytomas and another affected woman is said to have had bilateral adrenal tumors, although documentation of her adrenal pathology is unavailable. No C-kindred member is known to have parathyroid disease. We have studied one branch of the C-kindred since 1988. All patients in this study who have been treated surgically have had a total thyroidectomy and central node dissection.

The pentagastrin stimulation test consists of an intravenous bolus injection of pentagastrin (Peptavlon, Ayerst), 0.5 µg/kg body weight, with blood samples taken immediately before and 1, 2, and 5 minutes afterward.

The standard CT radioimmunoassay has been described (1,2). Upper limits of normal for basal- and pentagastrin-stimulated serum CT concentrations are 550 pg/mL and 750 pg/mL, respectively. The newer assays are a commercially available kit from Nichols Institute Diagnostics (San Juan Capistrano, CA) and a kit commercially available in Europe from International CIS (Gif-sur-Yvette, France). The Nichols assay is based on antiserum from a goat immunized with synthetic human CT (19) and is a competitive radioimmunoassay. The sensitivity for this assay is 1 to 3 pg/mL. The upper limits of normal for basal serum CT concentrations, as given by the manufacturer, are 36 pg/mL for males and 17 pg/mL for females. The upper limits of normal for serum CT concentrations after pentagastrin stimulation, as given by the manufacturer, are 106 pg/mL for males and 29 pg/mL for females. Basal serum CT concentrations were found to be above the lower limit of sensitivity in > 99% of our samples assayed with the Nichols kit. Most patients treated by total thyroidectomy had basal serum CT values of < 10 pg/mL by the Nichols assay with no change after pentagastrin injection.

The CIS assay is a sandwich assay using two monoclonal antibodies raised against a synthetic peptide with 1 to 32 amino acid sequence of the mature monomeric form of human CT (20,21). The sensitivity for the CIS assay is 6.7 pg/mL. Using the CIS assay, both normal males and normal females have basal values < 10 pg/mL and values up to 30 pg/mL after pentagastrin stimulation [(22) and manufacturer's package information]. In our studies, 39 (83%) of 47 clinically unaffected members of the J- and C-kindreds were found to have basal serum CT values below 6.7 pg/mL, and 44 (94%) of 47 had values < 10 pg/mL. Three males from unaffected branches of the J-kindred had slightly elevated basal serum CT concentrations of 16 to 39 pg/mL, but showed no rise after pentagastrin stimulation. The possible significance of these values is discussed later.

Patients with C-cell disease were considered to have a biochemical cure after total thyroidectomy when both their basal and peak pentagastrin-stimulated serum CT concentrations were less than 10 pg/mL, or when their basal CT level was between 10 and 20 pg/mL and less than a 50% increase occurred after pentagastrin stimulation. In some patients, the Nichols and CIS assays gave discrepant results, and only basal serum CT measurements were available in one case; the findings in these cases will be noted specifically.

Archived samples used for retrospective analysis were stored at –70 °C and had, in most cases, been thawed only once at the time of their initial measurements by the standard assay.

Results

Comparison of values obtained by the Nichols and CIS assays

CT concentrations by both of the high-sensitivity assays were compared in 227 serum samples taken from 60 pentagastrin stimulation tests in which the CIS assay gave a measurable value. Most of these samples were from known affected individuals, inasmuch as most known unaffected individuals had indeterminate values by the CIS assay. Values obtained with the Nichols assay were higher than those obtained with the CIS assay in 85% of samples. The mean ratio of Nichols:CIS values was 1.38, with 95% confidence limits of 1.22 to 1.56 for the mean. Values from both assays were highly correlated from < 10 to > 2,000 pg/mL, with $r = 0.845$ for log transformed values, $P < 0.0001$.

Sixty-seven samples were available in which serum CT concentration was measurable by the standard assay and in which values from the other two assays were determined. Using log-transformed data, the correlation between the values from the standard assay between 210 and 14,000 pg/mL and Nichols assay values was $r = 0.585$ ($P < 0.001$) and between standard assay and CIS assay values was $r = 0.615$ ($P < 0.001$). Values from the Nichols assay were up to 45% higher than those from standard assay in seven samples and were lower in the other 60. Values from the CIS assay were lower than those from the standard assay in all samples but one, in which the values were equal.

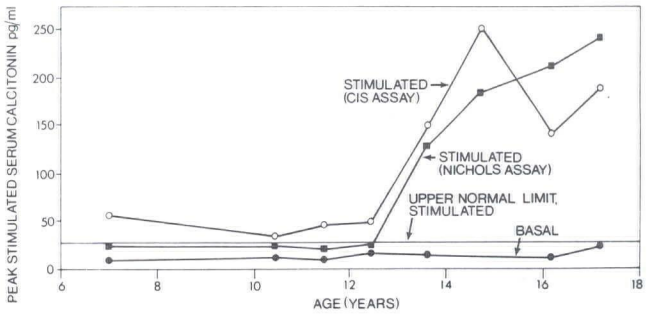
Clinical observations

CT measurements in preoperative serum samples—Between 1986 and 1989, three individuals were found to have abnormal CT secretory response results which revealed clinically important discrepancies among the assays. One of these (Figure) was a 17-year-old female who had been screened regularly since age 7 and had normal basal CT levels and pentagastrin-stimulated CT levels by the standard assay in every test. In archived serum, her basal and stimulated CT values by the Nichols assay were, in retrospect, normal through age 12½ years. Over the next year there was a sharp rise to an abnormal level in the stimulated CT value, measured by the Nichols assay, with further increases during the following three years. In the last test, the basal serum CT concentration by the Nichols assay was only minimally elevated (22 pg/mL), although the pentagastrin-stimulated level was as much as six times normal. Basal serum CT concentrations measured by the CIS assay (normal < 10 pg/mL) were slightly elevated in all available samples: the basal values were 15 pg/mL at age 7, 29 pg/mL at age 17, and between 10 and 35 pg/mL in the intervening tests (values not shown in the Figure). The stimulated-serum CT concentrations were also abnormal by the CIS assay in all of the available samples, but the elevations were minimal through age 12½. Like the Nichols assay, the CIS assay showed a marked rise in stimulated-serum CT levels thereafter. Total thyroidectomy was performed at age 17½; C-cell hyperplasia with several small medullary carcinomas, up to 5 mm in diameter, were found within the thyroid gland.

Two other patients had results similar to the patient illustrated in the Figure, although one had been lost to the study and had had no tests for five years before surgery. Retrospective measurements by the newer assays showed marked elevations of stimulated-serum CT concentrations for three to four years during which the standard assay had given normal values. Both glands showed C-cell hyperplasia and small bilateral foci of MTC.

Postoperative serum CT measurements—Individuals with pathologically proven C-cell disease were classified into three groups for analysis of the biochemical outcome of surgery.

Group I included 17 J-kindred and C-kindred members who had abnormal pentagastrin tests the first time they were tested (i.e., before prospective screening). The first tests in the J-kindred members used the standard assay, while the first tests in the C-kindred members used the Nichols assay. In this group, three patients died of causes unrelated to their MEN 2A diseases before our use of the Nichols and CIS assays. Of the other 14, four met criteria for biochemical cure, and 10 had clearly measurable postoperative serum CT concentrations by both the Nichols and CIS assays. In five of the 10, the basal serum CT concentrations were normal by both assays, with only the pentagastrin-stimulation test showing evidence of residual C-cell function (stimulated values between 36 and 450 pg/mL). One other of those with measurable serum CT postoperatively had advanced renal failure, and basal serum CT concentrations > 100 pg/mL, but less than a 50% increment after pentagastrin stimulation. Because renal failure is known to cause elevated serum CT concentrations in the absence of C-cell disease, the meaning of the elevated level in this patient is unclear. Thus, nine of 14 tested patients in group I did not achieve biochemical cure after total thyroidectomy and the status of one is uncertain, although only two had known anatomic sites of residual MTC. The median time between surgery and the most recent CT testing was 18 years (range 1 to 21 years).



Figure—Serum CT concentrations in a female J-kindred member measured in archived serum samples. Samples were stored at −70 °C for 1 month to 10 years before the measurements were performed. Stimulated values for each pentagastrin test are the highest of the values in the serum samples taken 1, 2, and 5 minutes after pentagastrin injection. The horizontal line indicates the upper limit of normal for stimulated values in females by the Nichols assay. The upper limit of normal for stimulated values by the CIS assay is 10 pg/mL. Solid circles and squares denote basal and stimulated values, respectively, by the Nichols assay. Open circles denote stimulated values by the CIS assay. For visual clarity, basal concentrations by the CIS assay are not shown, but are discussed in the text.

Group II included 12 J-kindred patients whose abnormality was found by prospective screening and who had at least one microscopic focus of MTC at the time of thyroidectomy. All were tested with the newer CT assays postoperatively, with a median time between surgery and the most recent CT testing of 13.5 years (range 1 to 16 years). Seven met the criteria for biochemical cure by both the Nichols and CIS assays. Two met criteria for biochemical cure by the CIS assay but had detectable pentagastrin-stimulated CT levels by the Nichols assay (18 pg/mL and 53 pg/mL). The other three had detectable basal- and stimulated-serum CT concentrations by both assays (basal values between 12 and 37 pg/mL, stimulated values between 33 and 293 pg/mL). Of the three patients who had evidence of C-cell disease several years before surgery when their archived serum samples were retrospectively tested, one had residual C-cell function according to the Nichols assay and the other two had residual C-cell function by both assays.

Group III included 13 J-kindred patients whose abnormality was found by prospective screening and who had only C-cell hyperplasia at the time of thyroidectomy. The median length of time between thyroidectomy and the most recent CT testing was 15 years (range 7 to 18 years). In group III, two patients were lost to follow-up, eight met criteria for biochemical cure by both assays, and two met criteria for biochemical cure by the Nichols assay but were not tested by the CIS assay. The remaining woman had a normal basal-serum CT concentration by both as-

says but did not receive pentagastrin because she was pregnant. Thus, in group III, no patient had evidence of abnormal residual C-cell function after surgery, although follow-up was incomplete.

Discussion

In this study, two high-sensitivity CT immunoassays were applied to the screening of individuals in MEN 2A kindreds for thyroid C-cell disease and results compared with studies using a standard assay. Both newer assays are capable of measuring serum CT concentrations 10- to 30-fold lower than the sensitivity limit of the CT assays available prior to about 1986, including the standard assay used in screening the J-kindred since 1969. The results obtained with the Nichols and CIS assays were qualitatively similar, though some quantitative differences were found. The Nichols assay gave results that were generally higher than those of the CIS assay. This finding is not surprising. The Nichols assay uses an antiserum raised against synthetic human CT, which may well recognize not only the intact form of CT but other molecular species as well. The CIS assay is designed to measure only the mature, amidated monomeric form of CT, which accounts for less than half of the total immunoreactive hormone in the circulation under basal conditions. The mature monomer predominates in the serum of patients with MTC after pentagastrin stimulation (23). The CIS assay detects CT in some patients who have renal failure or various neuroendocrine, lung, and other malignancies (24); CT precursors do not crossreact in the assay (25). Three unaffected men in our MEN 2A kindreds had slightly elevated basal CT levels by the CIS assay, with no significant rise after pentagastrin. All are clinically well, though they may conceivably have an undetected disease that raises the serum CT level, or have serum constituents, other than CT, that interfere in the CIS assay. It would appear that the standard CT assay recognizes more forms of the circulating hormone than either of the other methods.

Serum CT measurements in archived preoperative serum samples from MEN 2A patients with proven C-cell disease suggest that several affected individuals had developed abnormal C-cell function three to four years before detection by the standard assay. Similarly, Guilloteau et al (22), using the CIS assay to study one MEN 2A kindred, identified four cases of MTC in whom an older competitive radioimmunoassay method did not detect any abnormality. Thus, previous estimates of the age of onset of C-cell disease in MEN 2A may be too high by three to four years or more; the median age of onset may be closer to 8 or 9 years than 12 years (11,12). Consequently, negative test results by a high-sensitivity CT assay may provide more reassurance to a young adult than could be given with the previously available assays. For example, a 26-year-old individual with a normal pentagastrin test is probably closer to having a 5% risk of carrying the MEN 2A gene than the 10% risk previously estimated (11,12).

Another implication of the retrospective measurements is that the presence of intrathyroidal foci of MTC in about 50% of the J-kindred at the time of surgery is likely to reflect the fact that clear elevations in serum CT in the earliest stages of C-cell disease were not detected by the previously available assays. It is likely, therefore, that a higher percentage of patients will be identified in the premalignant phase of their C-cell disease when high-sensitivity CT assays are employed. Even with the increased sensitivity of the newer serum CT assays, it is still the case that pentagastrin stimulation reveals abnormal C-cell function up to several years before the basal serum CT concentration becomes abnormal (2-7).

Results from the high-sensitivity CT assays also modify previous conclusions (6) about the impact of total thyroidectomy on C-cell disease in MEN 2A. With the criteria we used for biochemical cure, group III individuals with pure C-cell hyperplasia treated by total thyroidectomy have an excellent cure rate. None tested thus far has evidence of residual abnormal C-cell function, though follow-up is incomplete. At the other extreme, only four of 14 group I patients, in whom MTC was present at initial testing before prospective screening began, met current criteria for biochemical cure. Since only one patient was cured of the three who died before we started using the newer assays, and one living patient has an uncertain status, the overall cure rate in this group is only about one-third. Previous estimates using the standard CT assay, supplemented by the Nichols assay in a few patients, were that half of the patients in this group were cured (6).

In group II patients, who had intrathyroidal MTC and C-cell hyperplasia detected by prospective screening, results of high-sensitivity CT testing suggest that a substantial minority of patients fail to achieve biochemical cure after total thyroidectomy. There were three or five such cases out of 12 depending on the significance attached to detectable stimulated-serum CT levels by the Nichols assay only. The reason that this conclusion differs from previously reported findings in the J-kindred (6) is that three of the five individuals in this group with evidence of residual C-cell function had their abnormality detected and the other two had their first abnormal follow-up tests after the previous analysis was completed.

The significance of postoperative residual C-cell function in relation to prognosis is not yet known. The group II patients who had detectable CT concentrations were 20 to 27 years old at the time of this analysis and had no other evidence of residual MTC. In the oldest generation of the J-kindred studied (diagnosed before any screening was started), five of seven affected individuals died of MTC, with ages at death of 51 to 69 years. Thus, diagnosis and treatment of C-cell disease in MEN 2A at the stage of C-cell hyperplasia appears to be the most effective management, but diagnosis and treatment in the stage of microscopic intrathyroidal MTC is probably advantageous also. However, an additional 40 years of observation may be needed to provide a direct test of this hypothesis.

On the basis of our findings, we recommend the use of high-sensitivity CT assays in MEN 2A screening programs. Admittedly, the frequency of a false-positive diagnosis when these tests are used is as yet unknown. Prospective screening is particularly demanding in this regard, because the goal is diagnosis early in the course of the disorder. Using the standard assay, there has been one definite false-positive diagnosis in 36 patients subjected to thyroidectomy in the J-kindred and three ad-

ditional patients (included in our group III) whose thyroid histopathology was mild diffuse C-cell hyperplasia but whose genetic linkage studies (16) predict a probability of less than 1% that they carry the MEN 2A gene (26). To date, seven untreated patients had abnormal pentagastrin tests by the newer CT assays. All had subsequent thyroidectomy; histopathology was microscopic MTC in four and diffuse C-cell hyperplasia in three. Thus, diagnostic accuracy of these high-sensitivity assays is excellent so far, but only a small number of patients have had surgery to date. The combination of genetic screening for the MEN 2A carrier state (15-18), when it becomes available, together with a high-sensitivity CT assay in prospective screening programs, should lead to greater accuracy in the preoperative diagnosis of C-cell disease than either method alone.

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