toring of CT levels might also be useful not only in the assessment of the treatment response, but serum CT and carcinoembryonic antigen (CEA) levels are also sensitive markers of tumor burden and dissemination.

Basal serum CT levels are also within the normal range in >90% of the first-degree relatives of patients with familial MTC and stimulation tests can help identify sub-clinical CCH (a pre-cancerous disease) in these subjects; the combined administration of Ca²⁺ and Pg has been suggested to be more sensitive in the latter subjects⁹.

We aimed to assess the role of serum CT stimulation tests (with Ca²⁺, Pg or their combination) in the identification of occult metastases in patients who had undergone surgery for MTC and in the early diagnosis of CCH in their relatives. We also recorded the type, severity and duration of side-effects during each stimulation test.

Material and Methods

Subjects and methods

We studied 10 patients who had undergone surgery for MTC and 20 first-degree relatives of theirs [18/30 females (60%), median age 35 years (range 18-52 years)]. Two of the patients and none of their relatives had multiple endocrine neoplasia (MEN) type 2A. Among the 10 patients with MTC, 4 patients had clinically apparent metastatic disease (cervical lymphadenopathy or pulmonary, liver or bone metastases) despite multiple operations and alternative antineoplastic treatments and died after 1, 3, 4.5 and 6 years of follow-up, respectively (Group 1A) (Table 1).

Two patients had initially minimal residual disease (very few and small, hardly palpable metastatic cervical lymph-nodes; Group 1B) that recurred after 2 operations and progressed slowly after 12 and 16 years of follow up. Finally, 4 patients had no residual disease (Group 1C) clinically or on usual imaging methods and did not relapse after a follow-up of 9, 11, 12 and 17 years, respectively. All patients had sporadic MTC except 1 patient from Group 1A and her son from Group 1B, who had MEN type 2A.

Patients older than 60 years were excluded from the study whereas younger patients with coronary heart disease, migraine, peptic ulcer or other systemic diseases underwent only the Ca²⁺ stimulation test.

We also studied 20 first-degree relatives of the 10 patients with MTC (Group 2) (Table 1). These 20 subjects had no clinical or imaging evidence of MTC or C-cell hyperplasia and had not previously undergone screening for the presence of the RET proto-oncogene. Four of these 20 subjects are relatives of the 2 patients with the MEN 2A syndrome.

All patients and all relatives provided written informed consent before undergoing any stimulation test.

Measurement of serum CT levels

Serum CT was determined with a radioimmunoassay (RIA-mat Calcitonin I, Mallinckrodt Diagnostica, 6057 Dietzenbach 2, Germany) with sensitivity < 0.078 pg/ml. Non-specific binding with this assay was < 5%, was determined during all stimulation tests and was accounted for when measuring CT levels. Intra-assay coefficient of variation (CV) was 11.3%, 4.9% and 5.8% at CT levels of 210, 670 and 1130 pg/ml, respectively. Mean intra-assay CV was 8.3% at CT levels of 1090 pg/ml.

All data were analyzed using the statistical package SPSS (version 13.0; SPSS Inc., Chicago, IL, USA) and are presented as medians and range.

Clinical protocol

The presence of clinically apparent or minimal residual MTC was assessed with clinical examination, ultrasonography, computed tomography and MRI (and PET in selected patients) and was confirmed with post-operative pathological examination. Metastases included multiple cervical lymph nodes bilaterally and/or nodular lung metastases who were identified during selective or modified lymph node dissection or with imaging studies.

Serum CT levels were measured at baseline and after stimulation tests with either a) Ca²⁺ 2 mg/kg infused i.v. within 50 sec b) Pg (Peptavlon, Ayerst) 0.5 μg/kg infused i.v. within 10 sec or c) Ca²⁺ 2 mg/kg (50 sec i.v. infusion) followed immediately by Pg 0.5 μg/kg (10 sec i.v. infusion) (combined stimulation test). Serum CT levels were determined immediately after the end of the infusion of the stimulating agents (time 0) and 1, 2, 3.5, 5 and 7 min later.

The combined stimulation test was performed in 25 of the 30 subjects and all 3 stimulation tests were performed in 14 of the 30 subjects.

Table 1: Groups of patients included in the study.

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>Characteristics</th>
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</thead>
<tbody>
<tr>
<td>1A</td>
<td>4</td>
<td>Patients with clinically apparent metastatic MTC</td>
</tr>
<tr>
<td>1B</td>
<td>2 10</td>
<td>Patients with minimal residual disease</td>
</tr>
<tr>
<td>1C</td>
<td>4</td>
<td>Patients without residual disease</td>
</tr>
<tr>
<td>2</td>
<td>20</td>
<td>First-degree relatives of patients with MTC without clinical or imaging abnormalities</td>
</tr>
</tbody>
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MTC: medullary thyroid carcinoma.