Detection of cervical metastases of thyroid medullary carcinoma by MoAb anti-CEA scintigraphy and immunohistochemistry

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Abstract. Four patients with medullary thyroid carcinoma (MTC) were examined using anti-carcinoembryonic antigen (CEA) scintigraphy. Two patients had positive and two normal scintigraphic findings, although all the patients had elevated blood test markers (calcitonin or CEA). One patient with clinical suspicion of MTC metastases had only a faintly positive anti-CEA image, although single-photon emission tomographic scanning was used to increase the sensitivity and resolution of the method. Therefore, digital image processing of the planar images was performed to obtain more detailed information. The analysis revealed distinct accumulation of the activity at the right side of the neck at 20 h post administration. The specificity of the antibody binding in the malignant cells was confirmed after surgery by immunohistochemical staining of the tumour specimens for CEA. Both conventional and confocal laser scanning microscopy revealed distinct positive staining, indicating that the results obtained from the anti-CEA scanning showed specific binding of the labelled antibody in the neoplastic tissue.

Key words: Anti-carcinoembryonic antigen scintigraphy—Medullary thyroid carcinoma—Single-photon emission tomography—Immunohistochemistry


Introduction

Medullary thyroid carcinoma (MTC) accounts for 2%–12% of all thyroid malignancies. MTC is a neuroendocrine tumour derived from the parafollicular C cells of the thyroid. It may occur in sporadic or, less commonly, familial forms, and in the latter case it may form part of the multiple endocrine neoplasia (MEN) syndrome. MTC tumours secrete calcitonin, which can be used as a marker of the disease. Positive immunohistochemical stains for calcitonin and carcinoembryonic antigen (CEA) are characteristic of the MTC [1]. Immunoassays for serum calcitonin and CEA are used for early detection of disease recurrence, but the localization of metastases is often difficult. MTC tumours are different from the other thyroid carcinomas in that they do not concentrate radioiodine and have a poor long-term prognosis.

Many different radiological techniques have been reported for imaging MTC. Radiolabelled anti-CEA monoclonal antibodies or antibody fragments, F(\(\text{Ab'}\))\(_2\), have been considered to offer the highest specificity and sensitivity in the scintigraphic imaging of MTC [2]. O’Byrne et al. [3] recently also demonstrated that indium-111 labelled anti-CEA F(\(\text{Ab'}\))\(_2\) scintigraphy, especially in conjunction with single-photon emission tomography (SPET), is useful for the diagnostic evaluation of patients with MTC. However, there are several problems with the anti-CEA scintigraphy techniques. These include non-specific uptake of the radiolabel in the spleen, kidneys, bone marrow and liver [3]. This case report demonstrates the results from four anti-CEA scintigraphic examinations, showing problems in the interpretation of the planar and SPET images. Digital image processing of the planar images was used in one of the present cases to demonstrate more clearly the metastases, which were barely distinguishable from the background accumulation in routine planar of SPET images.

Materials and methods

Patients

Four patients with sporadic MTC were investigated.

Patient 1. In 1981, a 52-year-old woman was found to have a thyroid nodule during a health examination. Histological examination after total thyroidectomy indicated that she had MTC. The tumour...
had not invaded the thyroid capsule and no evidence of macroscopic lymph node metastases was noted. No central dissection was performed. In 1992, she had elevated serum CEA and calcitonin levels: 6.7 μg/l (normal reference range<3 μg/l) and 1090 pmol/l (normal reference range<30 pmol/l), respectively. Further studies included cervical ultrasonography, cervicomediastinal CT scan and anti-CEA scintigraphy. The second cervical ultrasonographic examination, in 1993, showed pathological lymph nodes; thereafter she had a functional cervical dissection, and macroscopic lymph node metastases were found and excised. Her serum CEA and calcitonin levels subsequently normalized.

**Patient 2.** In 1973, a 25-year-old woman was found to have a thyroid nodule. At operation, the right lobe and isthmus were totally removed and a subtotal resection was done on the left thyroid lobe. Histological studies showed the tumour to be MTC. Thereafter, she received external cervical radiotherapy. In 1982, a cervical lymph node was removed, and it was shown to be a metastasis of MTC. During the same year, she received radioactive iodine ablation (100 mCi) to the remaining part of the left thyroid lobe. Additional cervical metastases were surgically removed in 1983 and 1985. Thereafter, her serum CEA level was normal and her serum calcitonin concentration varied between 37 and 41 pmol/l. In 1991, her serum calcitonin increased to 69 pmol/l and further studies, including a positive anti-CEA scintigraphy, were undertaken (Table 1). A functional cervical dissection was performed in 1992 and lymph node metastases of MTC were found and excised. Thereafter, her serum calcitonin level remained elevated at a level of 51 pmol/l and her serum CEA content was normal. The results of repeat anti-CEA scintigraphy 3 months after the operation suggested possible remnant cervical metastases. No re-operation has been performed.

**Patient 3.** In 1991, a 57-year-old woman was found to have a recurrent nerve palsy. Further studies indicated that she had two thyroid nodules. A total thyroidectomy was performed. At operation, the tumour was found to have invaded the thyroid capsule and to be adherent to the esophagus and the cervical muscles, but no enlarged lymph nodes were found and no central dissection was performed. Histological examination indicated that the tumour was MTC. One month later, cervical ultrasonography revealed enlarged cervical lymph nodes. A fine-needle biopsy indicated metastases of MTC. She received external radiotherapy by a linear accelerator and a betatron, after which her serum calcitonin level was 1130 pmol/l. A radical cervical dissection was performed and the MTC metastases were removed. Six months later her calcitonin level was elevated to 130 pmol/l and her serum CEA content was 3.3 μg/l. Despite this, cervical ultrasonography and anti-CEA scintigraphy did not indicate new metastases.

**Patient 4.** In 1984, during a health examination, a 50-year-old man was found to have a hard thyroid nodule, which he claimed to have been present for many years. A cervicomediastinal CT scan indicated that the tumour extended into the mediastinum. Cervical exploration was done, but only the isthmus was removed and the tumour was considered to be inoperable. The initial histological examinations were consistent with an anaplastic thyroid carcinoma. Later, a critical review of the histological data indicated that the tumour was, in fact, MTC. He received external cervical radiotherapy by a linear accelerator and a betatron. Thereafter, his serum calcitonin remained at a level of 9000-12000 pmol/l. In 1988, a radical cervical dissection was performed, but was found to be technically impossible. In 1993, ultrasonography indicated a cervical mass of 1.9x2.3x3.5 cm and anti-CEA scintigraphy was also found to be positive in the cervical region. After this examination, the patient had difficulty in swallowing and intense aching in the tumour region.

**Scintigraphic technique and digital image processing**

After labelling of the MoAb, 1 mg of intact IgG1 (740 MBq technetium-99m labelled MoAb 431/26; Behringwerke AG, Marburg, Germany) was injected over a period of 2-5 min. The anti-CEA scintigraphic examinations were performed using a Siemens Orbitom ZLC 37 gamma camera (Gamma Sonics, Inc., Ill., USA) equipped with a low-energy all-purpose collimator. Images were obtained about 10 min and 4 h post injection, with at least 50000 counts/planar image collected. Thereafter, SPET images were acquired in a 64x64 matrix in 6° steps, with rotation over 360°, 40 s per view. All the SPET images were processed with filtered back projection without attenuation correction. Transverse, coronal and sagittal slices with 1.25 cm thickness (two pixels) were produced. Twenty hours post injection another series of images was produced.

Digital image processing of the planar images from patient 2 was performed to enhance the contrast and code the intensity levels into colour for easier interpretation. The planar images (4 and 20 h post injection) of the head and the neck were studied on a light table, where they were viewed with a Videk Megapuls model 1400 CCD camera (Kodak Company, Canandaigua, N.Y., USA). The obtained grey-scale images were further studied using a MCID-M2 image analyser (Imaging Research Inc., St. Catharines, Ontario, Canada) with a spatial resolution of 636x508 pixels. The digitized images were converted from grey scale to pseudo-colour and displayed on a high-resolution colour monitor (Super Scan, Hitachi, Yokohama, Japan).

**Immunohistochemistry**

A sample of MTC from patient 2 was obtained alongside the routine histopathological specimens taken during surgery at the Oulu University Hospital. The tissue sample was dissected into small pieces of about 5 mm and immersion fixed in 4% neutral-buffered formaldehyde for 18 h at 4 °C and washed in phosphate-buffered saline (PBS) for 24 h at 4 °C. After rapid freezing in liquid nitrogen, the tissue specimens were stored at −80 °C. Tissue sections of 8 μm were cut for immunohistochemical staining using a Cryo-Cut Microtome (American Optical Corporation, Buffalo, N.Y., USA).

The tissue sections were stained for CEA using the immunofluorescence technique. The detailed staining procedure was as follows: The sections were first treated with swine serum for 40 min and then incubated with polyclonal rabbit antiserum to human CEA (Dakopatts, Copenhagen, Denmark) or a normal rabbit serum, diluted 1:50 in PBS containing 1% bovine serum albumin (BSA, Sigma Chemicals, St. Louis, Mo., USA). After washing for 3x10 min in PBS, the sections were incubated with rhodamine-conjugated swine anti-rabbit immunoglobulins (Dakopatts), which were also diluted 1:50 in BSA-PBS and then washed 3x5 min in PBS. All the incubation steps and washings were performed in a humidified chamber at room temperature. The sections were mounted in Mowiol prepared according to the instructions of the manufacturer (Hoechst, Frankfurt am Main, Germany), and viewed with a conventional microscope (Aristoplan, Leitz, Wetzlar, Germany) and a confocal laser scanning microscope (Leitz CLSM, Leica Laser Technics, Heidelberg, Germany) using argon-