Somatostatin-receptor scintigraphy in primary breast cancer

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Summary

Somatostatin-receptor (SS-R) scintigraphy successfully shows primary cancers and distant metastases in most patients with carcinoids, islet cells tumours, and paragangliomas. Previous in-vitro studies indicated that somatostatin receptors are present in human breast cancers.

We report positive scintigraphy with [^{111}In-DTPA-D-Phe^1]-octreotide in 39 of 52 primary breast cancers (75%). Parallel in-vitro autoradiography with [^{125}I-Tyr^3]-octreotide of 30 of these showed a corresponding somatostatin-receptor status in 28. Significantly more invasive ductal cancers could be shown than invasive lobular carcinomas (85% *vs* 56%; $p<0.05$). Also the number of T_2 cancers which were shown was higher than T_1 (86% *vs* 61%; $p<0.05$). Imaging of the axillae showed non-palpable cancer-containing lymphnodes in 4 of 13 patients with subsequently histologically-proven metastases. In the follow-up after a mean of 2·5 yr, SS-R scintigraphy in 28 of the 37 patients with an originally SS-R-positive cancer, was positive in the 2 patients with clinically-recognised metastases, as well as in 6 of the remaining 26 patients who were symptom-free. Raised carcinoembryonic antigent (CEA) and CA 15-3 values were observed in only 2 and 1, respectively, of these patients.

Most primary breast cancers can be shown by SS-R scintigraphy, especially invasive ductal cancers. This technique may be of value in selecting patients for clinical trials with somatostatin analogues or other medical treatments. Furthermore, SS-R scintigraphy is more sensitive than measurements of the usual serum cancer markers for detecting recurrences of SS-R-positive breast cancer.

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Introduction

Somatostatin receptors (SS-R) have been found in neuroendocrine tumours (carcinoids, islet cell tumours, paragangliomas) as well as in meningiomas.[1-5] Studies also show SS-Rs in some primary breast cancers,[6-9] probably of neuroendocrine origin.[8] Antiproliferative effects of somatostatin analogues have been reported on the growth of experimental cancers, including breast-cancer cell lines and explants.[10,11] We recently introduced a new technique in which SS-R-positive cancers could be shown in vivo, after giving radionuclide-labelled somatostatin analogue followed by gamma-camera scintigraphy.[12-16] In the present study we investigated this technique in primary cancers and metastates of 50 patients with breast cancer, and compared its value in the detection of recurrent disease with the serum markers CEA and CA 15-3, which are commonly used for this purpose.

Patients and methods

We studied 50 patients with 52 primary breast cancers (mean age 61; range, 38–93). After clinical examination and mammography, cancers were cytologically confirmed to be primary breast cancer. When patients gave informed consent to this study, blood samples were taken for measurements of cancer markers and SS-R scintigraphy was done as an outpatient. After scanning, all patients had an operation within 14 days, except for one, who had chemotherapy due to a T_4 breast cancer. Preoperative physical examination and chest X-ray showed no evidence of metastatic disease, nor were there clinical signs of local spread. 27 patients were treated by removal of the lump and axillary dissection, 22 with modified radical mastectomy (2 patients had a bilateral modified radical mastectomy). The Scarff, Bloom, and Richardson grade (SBR) was assessed by one pathologist (R v P) in all patients. The presence of somatostatin receptors in 30 of these cancers was measured by in-vitro autoradiography (J C R) on cryostat sections of cancer tissue, as has been described previously.[1,9]

The somatostatin analogue [DTPA-D-Phe^1]-octreotide (Mallinckrodt Medical BV, Petten, Netherlands) was labelled with ultra-pure ^{111}Indium.[18] Doses ranged from 200 MBq to 272 MBq [^{111}In-DTPA-D-Phe^1]-octreotide. Planar images were obtained with a large field-of-view gamma camera (Counterbalance 3700, Siemens Gammasonics, Erlangen, Germany) equipped with a 190-KeV parallel-hole collimator. Generally, the field of view covered the chest and the upper part of the abdomen. 24 hours after injection of [^{111}In-DTPA-D-Phe^1]-octreotide, chest images were obtained anteriorly and posteriorly, with additional images of the axillary region with arms elevated. 500 Kcts were collected per image with a maximum counting time of 15 min. A simple high/moderate, low/negative system was used to define the accumulation of radioactivity by the tumours as seen during scanning, carried out by E P K and H Y O, who were not informed where the cancer was.

Serum samples were stored at −20°C before assay. Serum CEA was determined by enzyme-linked immunoassay (ELISA) kits (Boehringer, Mannheim, Germany) (normal upper limit normal concentration 10 ng/mL). Serum CA-15·3 was determined by ELISA (Centeroor, Leiden, Netherlands).

Results

39 of the 52 tumours were seen on [^{111}In-DTPA-D-Phe^1]-octreotide scintigraphy. The intensity of scintigraphy varied considerably. In figure 1, the SS-R scan is shown of a 70-year-old with bilateral cancer and autoradiographic results obtained on a section of cancer removed from this patient, showing specific binding of [^{125}I-Tyr^3] octreotide. In 30 of these 52 cancers, autoradiographic studies of the surgically-removed tissue for the presence of SS-R could be done in parallel to the scintigraphy with [^{111}In-DTPA-D-Phe^1]-octreotide. SS-R's were present in 23 cancers; comparison between in-vivo scintigraphy and in-vitro autoradiography showed that receptors were found in both instances in 22, and absent on both investigatons in 6. A discrepancy between the in-vivo and in-vitro results was observed in 2. In one cancer, a non-homogeneously sparse distribution of SS-R's was found at autoradiography, while the tumour was not seen in vivo; in the other, low radioactivity was seen on the scintigram of the breast containing a SS-R-negative cancer according to autoradiography. Two types of SS-R distribution were recognised at autoradiography: in 16 the receptors were homogeneously and often densely distributed over the cancer tissue, while they were found to be non-homogeneously scattered in 7, all non-invasive ductal cancers.

Cancers showing a dense distribution of SS-R's in vitro were most clearly seen in vivo. Figure 2 shows the scan of a 56-year-old with a T_2 ductal carcinoma with a large non-invasive component. Autoradiography with [^{125}I-Tyr^3]-octreotide shows only specific binding of somatostatin throughout the non-invasive component of the tumour. Figure 3 shows the scintigram of a 39-year-old patient with a T_2 invasive ductal carcinoma of the left breast. Physical examination did not reveal palpable lymph nodes in the axilla. The scan, however, showed radioactivity in the axilla, and histology confirmed axillary lymph-node metastases. Of the subsequent 13 consecutive patients with histologically-proven non-palpable axillary

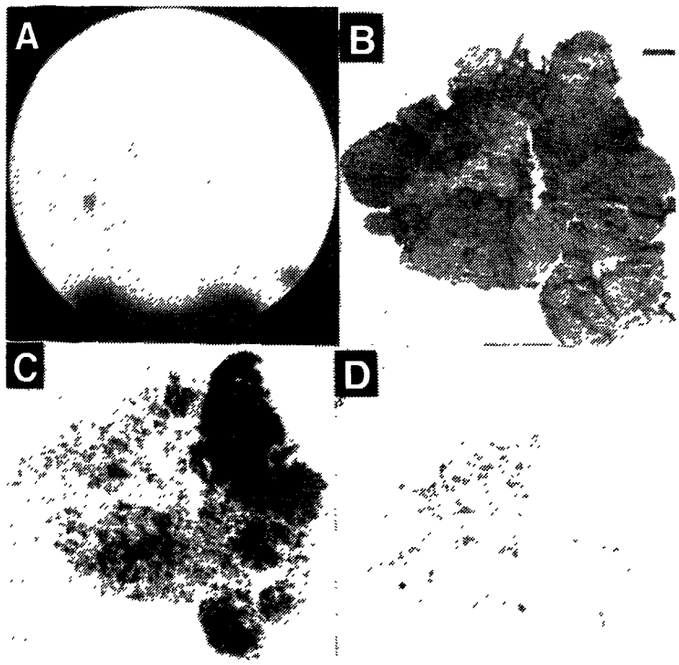

Figure 1: **A: [^{111}In-DTPA-D-Phe^1]-octreotide scintigraphy showing bilateral breast cancers. B: Haematoxylin-eosin stained section. C: Autoradiogram showing total binding of [^{125}I-Tyr^3]-octreotide. D: Autoradiogram showing non-specific binding of [^{125}I-Tyr^3]-octreotide (in presence of 10^{-6} M Tyr^3-octreotide).**

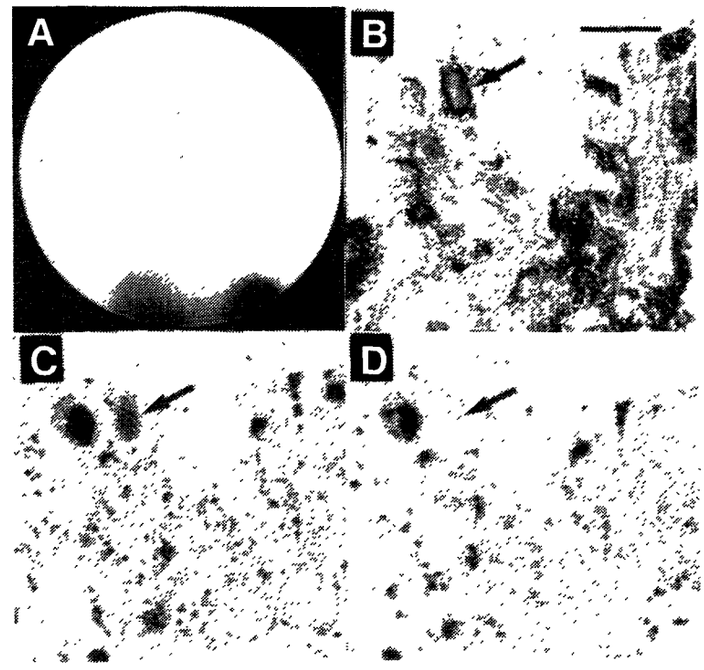

Figure 2: **A: [^{111}In-DTPA-D-Phe^1]-octreotide scintigraphy of a 56-year-old patient showing cancer faintly in the right breast. B: Haematoxylin-eosin stained sections containing non-invasive tumour tissue, ductal carcinoma-in-situ (DCIS). C: Autoradiogram showing total binding of [^{125}I-Tyr^3]-octreotide. Only one limited region containing DCIS is strongly labelled. D: Autoradiogram showing non-specific binding of [^{125}I-Tyr^3]-octreotide (in presence of 10^{-6} M Tyr^3-octreotide).**

lymph node metastases and a positive SS-R scan of the primary cancer, lymph-node metastases were seen in 4. None of the patients with a negative scan of the primary cancer showed abnormal radioactivity in the axillae or elsewhere in the body.

There was no correlation between [^{111}-In-DTPA-D-Phe^1]-octreotide positivity of the cancers in vivo and age (<60 yr 15/18 [83%]; >,60 yr 24/34 [71%]). 85% of the ductal cancers could be seen and 56% of the lobular cancers ($p < 0.05$). Also, significantly more T_2 cancers were seen than T_1 cancers ($p < 0.05$; table 1). 37 patients with 39 SS-R-positive primary breast cancers were selected to have repeat SS-R scintigraphy on average 2·5 yr after initial treatment (23–36 months). Of these patients, 2 had died (one due to metastatic breast cancer), 3 were bedridden at home (2 because of metastatic breast cancer), 2 had been discharged from follow-up, and 2 refused. Of the remaining 28 with an originally SS-R-positive primary breast cancer, 2 had already had repeat scintigraphy because of suspected metastases. In one of these, SS-R-positive metastases had been shown in the liver, lung, and cervical spine; and in the other, both axillary and mediastinal lymph-node metastases

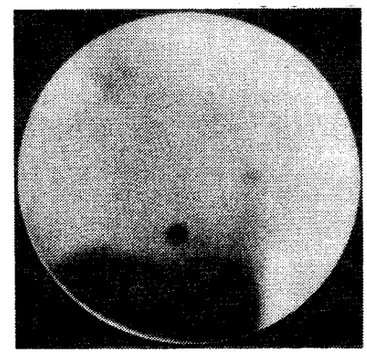

Figure 3: **[^{111}In-DTPA-D-Phe^1]-octreotide scintigraphy of a 39-year-old patient showing breast cancer on the left side and axillary-node metastases.**

	No of cancers	Positive SS-R scan (%)
Cancer size		
T_1	23	14 (61%)
T_2	28	24 (86%)*
T_4	1	
Histological stage		
T_1N_0	15	9 (60%)
T_2N_0	14	12 (86%)
T_1N_1	8	5 (62%)
T_2N_1	9	8 (89%)
T_2N_2	5	4 (80%)
T_4N_2	1	1

* < 0 05 *vs* T_1.

Table 1: **[^{111}In-DTPA-D-Phe^1]-octreotide uptake in primary breast cancer related to cancer size and stage**

were seen on the side of the original breast cancer. 26 patients were symptom-free; physical examination and scintigraphy showed lesions suspected to be metastases which were confirmed by biopsy, bone scan, ultrasound, or computerised tomographic scan: bone (4); liver (1); pulmonary (2); pleural (1); and in axillary (2), infraclavicular (1), and mediastinal (1) lymph nodes. In one patient, treated by cancer removal and axillary dissection, a local recurrence was seen at SS-R scintigraphy (figure 4).

14 patients with an originally SS-R-positive primary cancer, had a normal somatostatin scan on follow-up. Slight scattered radioactivity distributed over one lung was seen in 6 patients after radiotherapy following by cancer removal and axillary dissection. The 17 with an originally SS-R-negative scan were not rescanned; none have died and all are symptom-free.

Serum CA 15-3 and CEA were normal at first presentation in 35 and 37 patients respectively, with a SS-R-positive primary cancer and none of these cancer markers was raised in patients with primary SS-R-negative tumours, of which the SS-R status is based on SS-R scintigraphy. At follow-up, only one of the symptomatic patients had raised CA 15-3 and CEA, and also 1 of the asymptomatic patients had increased CA 15-3. All patients

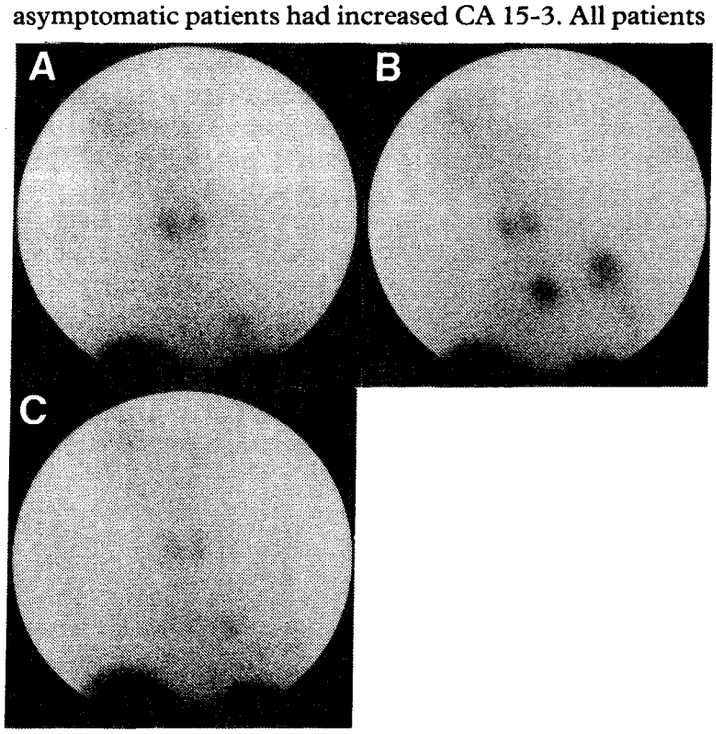

Figure 4: **A: 58-year-old patient with primary breast cancer of the left breast. B: 17 months later, mediastinal and axillary lymphnode metastases were shown on the left side. Treatment with chemotherapy followed. C: Thirteen months later there was complete disappearance of the axillary cancer and a partial response of the mediastinal cancer infiltration**

	CA 15-3 (U/mL)		CEA (ng/mL)	
	Admission	Follow-up	Admission	Follow-up
Symptomatic (n=2)	17	23	2	2
	17	331	1	30
Asymptomatic (n=6)	11	14	1	1
	42	92	1	2
	11	11	1	1
	22	20	1	1
	22	22	8	8
	25	21	2	2

Symptomatic: patients with clinically-overt metastatic disease.
Asymptomatic: patients without clinically-overt metastatic disease.

Table 2: **CA 15-3 and CEA in patients with SS-R-positive primary breast cancers and proven recurrent disease according to SS-R scintigraphy at follow up of 30 months**

with a normal SS-R scan at follow-up had serum cancer-marker values in the normal range except for 1 who had a slightly elevated CA 15-3 as was the case at first presentation (table 2).

Discussion

SS-R imaging has been shown to be successful in the identification of primary as well as metastatic cancer sites of a variety of neuroendocrine tumours.[12-16] Validation of the technique was by in-vitro demonstration of high-affinity binding sites for somatostatin in those cancers, which had been shown in vivo.[14] A positive SS-R scan closely predicted a beneficial effect of octreotide treatment on hormonal hypersecretion by these tumours.

Some breast cancers contain SS-Rs measured in vitro. In a group of 158 small breast-cancer samples (mean section surface 14 mm^2) 34 (21%) were SS-R positive, while in a group of 72 larger cancer samples (mean section surface 180 mm^2) 33 tumours (46%) were SS-R positive.[6] A subpopulation of SS-R-positive breast cancers is probably of neuroendocrine origin.[8,17] The percentage of SS-R-positive cases varies not only with the specificity and sensitivity of the neuroendocrine markers used, but depended also on the number of tissue slices per cancer investigated microscopically.[8] We showed 39 of 52 primary breast cancers (75%) to be somatostatin-receptor positive by scintigraphy. There was a close correlation between the in vivo [^{111}In-DTPA-D-Phe^1]-octreotide scan and subsequent in vitro autoradiography with ^{123}I-Tyr^3-octreotide. There was variability in radioactivity at SS-R scintigraphy; higher density in vivo correlated mostly with homogeneous and dense distribution of SS-Rs at autoradiography, while lower density of radioactivity over the cancer area in vivo corresponded with a non-homogeneous and sparse distribution of these receptors in vitro. Interestingly, low density of receptors seemed to be due to a non-invasive cancer component, mainly ductal carcinoma-in-situ.

The high incidence of SS-Rs in these 52 cancers, as observed by scintigraphy might be due to several causes. Firstly, in-vivo SS-R demonstration of breast cancer may be more sensitive than in-vitro autoradiography, as scintigraphy shows the presence of receptors in the whole cancer. In accordance with this, statistically significantly more T_2 than T_1 cancers were seen in vivo. Secondly, our patients might represent a selected group in comparison with those from other countries, as the incidence of ductal cancers amongst patients with newly-diagnosed breast cancers has increased over the last years in the Netherlands following the introduction of routine and repeated screening of the population.[19] Little is known about the

biological behaviour of SS-R-positive breast cancer, although a retrospective study of 110 patients[20] suggested that the presence of SS-R might predict a longer disease-free survival. Also, in-vitro studies of more than 300 breast-cancer samples showed an inverse relationship between somatostatin and EGF-receptor expression.[6,9] These observations suggest that patients with SS-R-positive cancers might have a relatively good prognosis. This, however, is not substantiated by our observations. After a mean follow-up of 2·5 yr we found that of 37 SS-R-positive patients 5 had extensive metastases, and also 6 of 26 symptom-free patients had metastases initially shown by SS-R-receptor scintigraphy.

CA 15-3 and CEA are the most commonly-used cancer markers to monitor patients with recurrent breast cancer. Both were raised in only 5–20% of women with primary breast cancer, but elevations between 61% and 84% have been recorded for women with extensive metastatic disease. CA 15-3 seems to be related to the extent of the metastases, the number of metastatic sites and survival, whereas CEA is only correlated with the extent of disease.[21,22] We show a higher sensitivity of SS-R scintigraphy compared with these cancer markers in detecting the development of recurrence in patients with SS-R-positive primary breast cancer. SS-R scintigraphy showed recurrent disseminated breast cancer in 6 (only 1 of whom had symptoms of recurrence) out of 28 patients with SS-R-positive primary cancers; all 6 patients had normal CA 15-3 and CEA. Another 3 out of these 28 patients had abnormal serum cancer markers, 2 of whom had an abnormal SS-R scintigram. The 3rd patient, who only showed marginally elevated CA 15-3 both at first presentation and follow-up, is clinically in remission 3·2 yr after the operation. Peptide-receptor demonstration of primary breast cancer with a radionuclide-labelled somatostatin analogue is successful in 75% of cases.

At primary diagnosis, the scintigraphic technique seems of minor value in the detection of axillary lymph node metastases; detection of these may be improved with guided surgery using a hand-held radio-nuclide detecting probe after administration of a radionuclide-labelled somatostatin analogue. The results so far also suggest that distant metastases of the primary somatostatin-receptor breast cancers continue to express such receptors at a mean of 2·5 yr after initial treatment. Scintigraphy showed the unexpected presence of metastases in nearly 25% of symptom-free initially SS-R-positive breast cancer patients, who had normal CA 15-3 and CEA serum values. The clinical relevance of detecting recurrent disease in an early stage is not known.

In-vitro studies with breast-cancer cell lines indicate a direct receptor-mediated inhibitory effect of somatostatin analogues on cell proliferation.[10,23-25] No prospective, controlled studies on the use of somatostatin analogue in patients with breast cancer have yet been published. As SS-R scintigraphy is effective in the early detection of SS-R-positive breast cancer recurrence, it may have future use in the selection of patients who can be treated with somatostatin analogues or radiotherapy with an α-emitting or β-emitting radionuclide coupled to a somatostatin analogue.

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