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The Relevance of Somatostatin Receptors in Thyroid Neoplasia

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^{111}In-octreotide scintigraphy in patients with persistent medullary thyroid carcinoma (MTC) visualized tumors in about half of the surgically explored sites. Tumor visualization correlated with rapid tumor growth and large tumor volume as judged from calcitonin levels. The ^{111}In concentration ratio between tumor (T) and blood (B) in surgically excised lymph node metastases of MTC showed a large variation, with low values for microscopic and high values for macroscopic metastases in individual patients. Three cases of MTC, Hürthle cell adenoma and papillary thyroid cancer are reported with preoperative scintigraphy, T/B ratios and Northern analyses of the surgical biopsies. Visualization of tumors was possible in the absence of sstr2 (the high affinity receptor for octreotide) with the exception of microscopic tumor growth. T/B values in the patient with Hürthle cell adenoma were similar to those found in the contralateral thyroid lobe with goitre. The relatively high uptake of ^{111}In in benign thyroid conditions probably limits the use of octreotide scintigraphy in the diagnosis of primary tumors. The technique has certain advantages over radioiodine scintigraphy after the surgical treatment of thyroid tumors: no need for withdrawal of thyroxin substitution; a possibility to diagnose metastases of tumors that do not concentrate radioiodine (MTC, Hürthle cell cancer); and complementary information about metastatic sites of non-medullary thyroid cancer (papillary and follicular tumors).

INTRODUCTION

Neuroendocrine tumors, including medullary thyroid carcinoma (MTC)[e], may express somatostatin receptors (sstr). Somatostatin and its long-acting analogue, octreotide, have been shown to inhibit incorporation of ^{3}H-thymidine into human thyroid follicular cell lines indicating expression of sstr physiologically. Somatostatin inhibits DNA synthesis induced by thyrotropin in such cell lines [1, 2]. However, after neoplastic transformation, somatostatin analogues may induce proliferation as shown in differentiated human thyroid cancer cell lines [3]. In patients with MTC, the only effective treatment today available is surgery [4]. In cases of differentiated non-MTC tumors, distant metastases can be treated with radioiodine. Radioiodine can also be used to diagnose residual thyroid cancer in the neck as well as distant metastases. However, one third of the non-MTC tumors do not accumulate radioiodine [5]. One future aim will be to investigate expression of sstr on various types of thyroid tumors to be able to evaluate their uptake of a radiolabelled somatostatin analogue, ^{111}In-octreotide, for diagnostic and therapeutic purposes.

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[e] *Abbreviations:* MTC, medullary thyroid carcinoma; sstr, somatostatin receptor; CEA, carcinoembronic antigen, ROI, region of interest; T, tumor; B, blood; Ti, tissue; Bg, background.

MTC is a neuroendocrine tumor derived from thyroid C-cells. In patients with MTC, basal and stimulated serum calcitonin concentrations are sensitive tumor markers reflecting the tumor volume [6, 7]. Presence of lymph node metastases in MTC has a more serious impact on survival than in papillary thyroid carcinoma [8]. One third of the MTC patients with persistent, or recurrent, disease after primary thyroidectomy can have their calcitonin levels normalized after repeat meticulous neck operations with microdissection technique [4]. Complete work-up with ultrasound, CT, MR and bone scintigraphy has low sensitivity in the localization of new lesions [9]. The surgical results would improve if good tumor localization methods were available. Selective catheterization and calcitonin determinations can often localize tumors to a region, but not to a specific site. Scintigraphic methods utilizing ^{123}I- or ^{131}I-MIBG, ^{201}Tl-chloride, ^{99}mTc-V-DMSA or radiolabeled monoclonal antibodies against calcitonin or carcinoembronic antigen (CEA) have been of limited value so far [10]. In a previous study, we found that octreotide scintigraphy visualized large primary MTC tumors, but only half of the surgically identified neck metastases [11].

In the present study, the ^{111}In concentration ratios between tumor and blood (T/B) were determined and compared with the findings at ^{111}In-octreotide scintigraphy in three different thyroid tumor forms (MTC, Hürthle cell adenoma and papillary thyroid cancer). In these individual patients, Northern analyses of all five sstr subtypes were also performed.

MATERIAL AND METHODS

Patients

Fifteen patients had previously been thyroidectomized for MTC. They were followed with annual tests for serum concentrations of calcitonin and CEA. At the time of the present investigation all patients had raised calcitonin concentrations indicating persistent disease. Before repeat surgery, including meticulous lymph node dissection, they underwent scintigraphy after injection of ^{111}In-DTPA-D-Phe^1-octreotide. After the operations, the ^{111}In concentration ratios were determined between freshly harvested lymph node metastases and blood.

Three patients with different types of thyroid tumors were worked up in detail with preoperative scintigraphy and determinations of T/B ratios and sstr subtypes: Patient A had previously been subjected to thyroidectomy due to MTC and had persistent disease; Patient B had a Hürthle cell adenoma in a nodular goitre; and patient C had a papillary thyroid carcinoma with lymph node metastases.

Scintigraphy

The patients received 190-350 MBq ^{111}In-DTPA-D-Phe^1 octreotide (10-20 μg) (Mallinckrodt, Petten, the Netherlands) by i.v. route. The amount of ^{111}In bound to octreotide was higher than 98 percent. Imaging was done 24-48 hr after injection of the radiopharmaceutical. A gamma camera (GE 400 AC/T), equipped with a medium energy parallel hole collimator and connected to a GE STARCAM computer system, was used. Data acquisition was done in a dual window setting (173 and 247 keV). Single photon emission computed tomography (SPECT) was performed in all patients (see [12] for further details).

The ratio between tumor (T) to background (Bg) uptake was calculated from preoperative scintigraphic images. A region of interest (ROI) was drawn over the tumor to estimate the activity content of the tumor and the same ROI was used to estimate the activity

content of the background (normal thyroid if present, otherwise tissue adjacent to the tumor).

Calcitonin and CEA assays

Serum calcitonin was determined by a non-extraction immunoradiometric assay with a detection limit of 2.4 ng/l (Medgenix, Fleurus, Belgium). The upper normal reference limit after thyroidectomy was 20 ng/l. CEA was determined with an immunofluorometric assay (Delfia, Turku, Finland) with a detection limit of 0.2 µg/l and upper normal reference limit of 5 µg/l.

Measurement of ^{111}In concentration in tissue samples and blood

Before histopathological examination, the fresh surgical specimens and blood samples withdrawn during surgery were weighed, and the ^{111}In concentration was measured in a calibrated gamma counter equipped with a NaI(Tl) well crystal (diameter 7.6 cm, length 7.6 cm, Harshaw, De Meern, Holland) and a single-channel pulse-height analyzer (Elscint, Haifa, Israel). Corrections were made for background activity and radioactive decay. Tissue-to-blood ^{111}In concentration ratios, (Ti/B), were calculated as well as T/B ratios, for all histology-proven biopsies [cf. 13].

Northern analyses of sstr1-5 mRNA expression

Fresh tumor specimens were rapidly frozen in liquid nitrogen. RNA was prepared by acid guanidine thiocyanate-phenol-chloroform extraction [14]. Samples of total RNA (20 µg) were heat-denatured and electrophoresed in a one percent agarose gel with 2.2 mol/l

Table 1. ^{111}In concentration between tumor and blood (T/B) in lymph node metastases of medullary thyroid carcinoma at various time intervals after injection of ^{111}In-DTPA-D-Phe^1-octreotide in 15 patients with persistent disease.

Time after injection	Pat. no.	T/B	Number of metastases
1 day	I	4-39	9
	II	2*-41	10
2 days	III	9	1
	IV	4-33	6
	V	17-47	5
5 days	VI	15-18	4
	VII	11-24	2
	VIII	3-43	2
	IX	36	1
	X	4-13	3
	XI	3*-240	9
6 days	XII	2*-32	16
7 days	XIII	3*-350	5
	XIV	6-14	7
	XV	27-34	2

* Microscopic tumor growth.

formaldehyde, 1 mmol/l EDTA, 5 mmol/l sodium acetate and 20 mmol/l morpholine propane sulphonic acid (pH 7.0) as running buffer. RNA was transferred to positively charged nylon membranes (Boehringer, Mannheim, FRG) using a vacuum transfer system (Hybaid, Middlesex, UK) and crosslinked to the membrane by UV-light (Stratalinker, Stratagene, La Jolla, CA). Membranes were hybridized in rotating flasks at 65°C (Hybaid, Middlesex, UK) using ^{32}P-labelled antisense RNA probes. The following cRNA probes were used:

1. A 1.126 bp PCR fragment of the human sstr1 corresponding to nucleotides 352-1478 [14].
2. A 1.7 kb Bam HI/Hind III cDNA fragment of the human sstr2 [15].
3. A 1.9 kb Nco I/Hind III cDNA fragment of the human sstr3 [16].
4. A 2.0 kb Nae I/Xba I cDNA fragment of the human sstr4 [17].
5. A 1.6 kb Eco R1/Sal II cDNA fragment of the human sstr5 [18].

Specific labelling was detected after three to six days exposure on an imaging plate, followed by reading in a Phosphor Imager (Molecular Dynamics, Sunnyvale, CA).

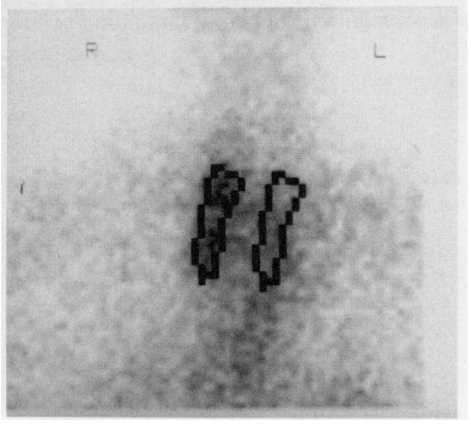

Figure 1. ^{111}In concentration ratio between tissue biopsies and blood (Ti/B), between tumor and adjacent tissue from scintigraphy (T/Bg) and somatostatin receptor subtypes in patient A with recurrent lymph node metastases in the neck after total thyroidectomy for MTC (cf. Pat no IV in Table 1), two days after injection of ^{111}In-DTPA-D-Phe^1-octreotide.

Histopathological findings	Ti/B (biopsy)	T/Bg (scintigraphy)	Receptor subtypes
Pos. lymph node	31		
Pos. lymph node	32	1.2*	sst_1, sst_{4-5}
Pos. lymph node	33		
Neg. lymph node	3.8		
Fat	5.7		

* Bg, adjacent tissue.

RESULTS

T/B ratios of lymph node metastases in patients with persistent MTC

The T/B ratio for the lymph node metastases, excised at various time intervals (1-7 days) after injection of ^{111}In-DTPA-D-Phe^{1}-octreotide in the 15 patients, showed a wide intra- and interindividual variation (Table 1). In the entire material, the lowest T/B values were seen in nodes with microscopic tumor growth. In four patients (I, II, XII and XIV), T/B values were plotted against the weights of the dissected lymph nodes for each individual patient. No significant correlation was demonstrated. Some patients had metastatic lymph nodes with high T/B values beside nodes with microscopic growth and low T/B values (Table 1). Patient no. XI had five out of nine nodes with T/B ratios exceeding 80. The highest value of 240 was seen in a medium-sized tumor. Patient no. XIII had three out of five nodes with values exceeding 190. The highest value of 350 was seen in the smallest tumor.

Patient A (persistent MTC)

Female, born 1957, with total thyroidectomy and neck dissection in 1987. During 1990-1993 she had three further neck operations due to fast growing recurrent tumors. In 1995, she again had increasing tumor markers. Before surgery, ^{111}In-octreotide scintigraphy suggested the presence of right-sided lymph node metastases (Figure 1). Three excised lymph node metastases with macroscopic tumor growth had five- to eight-fold higher T/B ratios than normal lymph nodes, or fat tissue samples. The ratio of T/Bg uptake calculated from the preoperative scintigraphic images was much lower than the T/B ratios (Figure 1). Northern analyses of a lymph node metastasis revealed expression of all receptor subtypes except sstr2 and sstr3 (Figure 4). Repeat surgery, guided by the scintigraphy, resulted in a 70 percent reduction of calcitonin levels.

Patient B (Hürthle cell adenoma)

Female, born 1925, with left-sided thyroid nodule. Fine needle biopsy suggested the presence of a malignant tumor. Preoperative ^{111}In-octreotide scintigraphy visualized the entire thyroid gland with more intense uptake over the adenoma (Figure 2). The ratio of T/Bg uptake calculated from scintigraphy was lower with a thyroid background than with a background of adjacent tissue. A total thyroidectomy was performed with lymph node dissection. The histopathological examination demonstrated a Hürthle cell adenoma. The remaining parts of the thyroid gland revealed a nodular goitre with areas of severe sclerosis. The T/B ratio for the adenoma was higher than for the pyramidal and contralateral thyroid lobe. The lymph nodes had much lower values and had a normal histological appearance (Figure 2). Northern analyses of the adenoma revealed expression of all five receptor subtypes except sstr2 (Figure 4).

Patient C (papillary thyroid carcinoma)

Male, born 1918, with bilateral thyroid tumors. Fine needle biopsy indicated papillary thyroid carcinoma. Preoperative ^{111}In-octreotide scintigraphy visualized bilateral uptakes in the thyroid (Figure 3). The T/Bg ratio from scintigraphy did not reveal any differences between the two tumors, while the T/B ratio for the left-sided lesion was twice as high as for the right-sided lesion. A total thyroidectomy was performed with neck dissection. The histopathological examination demonstrated bilateral papillary thyroid carcinoma as well as a thyroid cyst. Of the lymph node metastases, one was clinically overt and three were microscopic. Twelve lymph nodes were tumor-free. The T/B values for the tumor were higher than the Ti/B value seen for normal thyroid tissue and for the thyroid cyst. The lymph node with macroscopic growth had similar values as two of the three nodes with

microscopic growth. These values were not significantly different from the tumor-free nodes (Figure 3). Northern analysis of the papillary carcinoma revealed expression of all five receptor subtypes except sstr2 (Figure 4).

DISCUSSION

In a previous study of MTC-patients [11] with biochemically proven persistent disease, ^{111}In-octreotide scintigraphy visualized lymph node metastases in about half of the patients in accordance with other investigators [10, 19]. These patients had significantly higher basal concentrations of serum calcitonin and CEA indicating larger tumor volume. This finding was confirmed by morphometric studies of lymph node metastases. Kwekkeboom et al. [20] had previously shown that small MTC tumors, although expressing sstr shown by autoradiography, were not always visualized scintigraphically. They could not decide whether this was due to small size of the lesion or to low density of sstr, or both. The growth rate of MTC can be monitored by measuring the increase of serum calcitonin with time [7, 21]. There is an evident relation between the increment of plasma calcitonin and bad prognosis and tumor recurrence [21]. In our own series, the annual increment of basal calcitonin levels was much higher in patients with scintigraphically positive tumors. This finding could be due to large tumor volume and/or expression of sstr in the rapidly growing tumors. In our series, tumor-associated symptoms and mortality

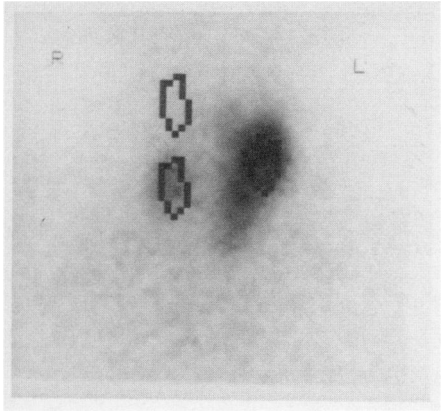

Figure 2. ^{111}In concentration ratio between tissue biopsies and blood (Ti/B), between tumor and adjacent tissue from scintigraphy (t/bg) and somatostatin receptor subtypes for patient B with unilateral Hürthle cell adenoma in nodular goitre, five days after injection of ^{111}In-DTPA-D-Phe^1-octreotide.

Histopathological findings	Ti/B (biopsy)	T/Bg (scintigraphy)	Receptor subtypes
Primary tumor (sin)	45	1.7* 3.4**	sst_1, sst_{3-5}
Contralat. lobe (dx)	32		
Pyramidal lobe	34		
Neg. lymph nodes	2-13 (n = 23)		

* Bg, thyroid
** Adjacent tissue

during the period of follow-up occurred only in patients with positive ^{111}In-octreotide scintigraphy [11]. These results are in contrast to the study performed by Kwekkeboom et al. [20], in which the ratio between basal serum calcitonin and CEA concentrations were high in patients with positive imaging. They interpreted this finding that well-differentiated tumors expressed sstr, especially since one MTC tumor, previously studied in several tumoral regions by autoradiography, seemed to express sstr in the more differentiated parts.

In a previous study, we studied T/B ratios in several different scintigraphy-positive neuroendocrine tumors with large variations between tumor types: low values for MTC (3-39), high values for carcinoid tumors (27-650) and sometimes very high values for endocrine pancreatic tumors (910-1500) [13, 22]. The T/B ratio seemed to be little influenced by the time interval between injection of ^{111}In-octreotide and surgery. This indicates

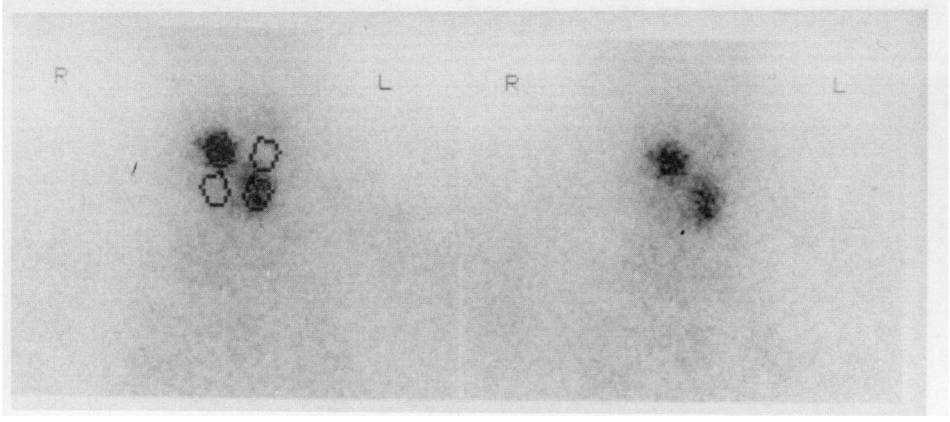

Figure 3. ^{111}In concentration ratio between tissue biopsies and blood (Ti/B), between tumor and adjacent tissue from scintigraphy (t/bg) and somatostatin receptor subtypes for patient C with bilateral papillary thyroid carcinoma with lymph node metastases, five days after injection of ^{111}In-DTPA-D-Phe^1-octreotide.

Histopathological findings	Ti/B (biopsy)	T/Bg (scintigraphy)	Receptor subtypes
Primary tumor sin	54	2.5**	sst_1, sst_{3-5}
Primary tumor dx	27	2.7**	
Pos. lymph node	10		
Pos. lymph node	10*		
Pos. lymph node	9*		
Pos. lymph node	1.4*		
Normal lobe sin	10		
Thyroid cyst	6		
Normal lobe dx	10		
Neg. lymph nodes	4,4,4,7,7,7,8,8,10,15,16,22 (n = 12)		
Fat	1.2		

* Microscopic tumor growth.
** Bg, thyroid.

similar pharmacokinetics ^{111}In in tumor and non-tumor tissues, including blood, after the initial uptake phase [23]. In some studies using radiolabelled monoclonal antibodies on experimental tumors, a declining T/B ratio has been shown with increasing tumor size [24]. These findings have not been corroborated in patients with neuroendocrine tumors receiving ^{111}In-DTPA-D-Phe^{1}-octreotide [13], or in the present patients with MTC. Part of the discrepancy in T/B between carcinoids and MTC may be due to lower tumor volumes in MTC, where early recurrence is diagnosed by sensitive biochemical methods. Other factors may be different proportions of tumor cells in the biopsies, and variable expression of

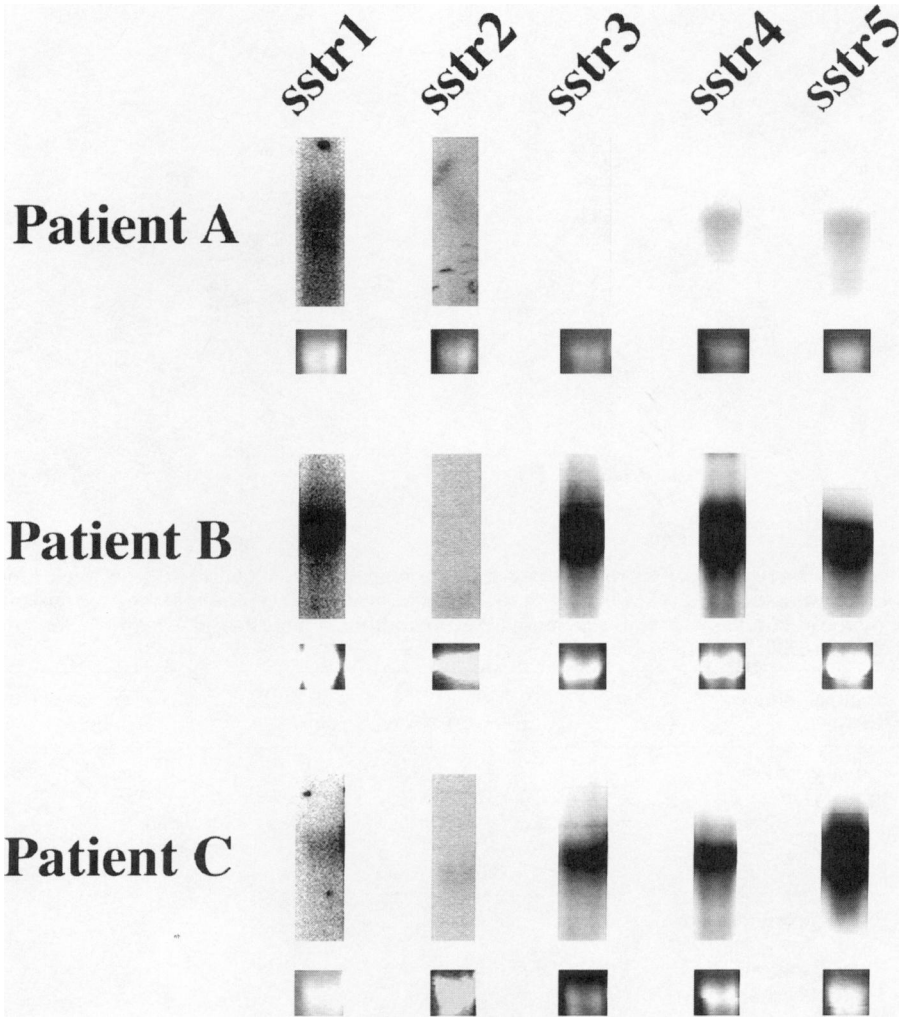

Figure 4. Northern blot analyses of sstr1-5 in tumor material from three patients: Medullary thyroid carcinoma (A), Hürthle cell adenoma (B) and papillary thyroid cancer (C). All patients lacked expression of sstr2, and patient A also lacked expression of sstr3. 18 S ribosomal bands serve as controls.

different sstr subtypes. These factors may also explain the discrepancy between patients and between tumor tissues from individual patients with MTC (Table 1). Octreotide has the highest binding affinity for sstr2, followed by sstr5 [25]. In patients, A-C sstr2 was not revealed by Northern analysis, even though the tumors were scintigraphically visualized most likely due to expression other sstr. Patient A also lacked expression of sstr3. No quantitative studies on receptor densities for these tumors are available.

In a previous study of 21 non-medullary thyroid cancers (papillary, follicular and anaplastic), ^{111}In-octreotide scintigraphy visualized specific uptakes in all patients with primary tumors and in 75 percent of the metastatic lesions. The examination had a complementary value to radioiodine scintigraphy, especially in the detection of skeletal metastases, while radio-iodine seemed to be superior in detecting pulmonary metastases. No obvious difference in sensitivity occurred between follicular and papillary tumor types. After withdrawal of thyroxine in preparation for radioiodine scintigraphy, the uptake of ^{111}In-octreotide appeared to be increased, possibly indicating sstr-upregulation by thyrotropin [26].

The clinical value of octreotide scintigraphy in patients with recurrent MTC is its potential for tumor localization. This can be of importance in patients, who have been subjected to several neck exploration with severe scar tissue formation. In these patients, directed surgical explorations can be of substantial value (cf. Patient A). However, it is important to bear in mind that only half of the surgically found metastases were visualized in the receptor-positive patients [10, 11], which means that the surgical exploration should be extended beyond the visualized sites. Visualization of lymph node metastases may also serve as an indicator of worse prognosis [11].

Hürthle cell adenoma/carcinoma are thyroid tumors that cannot be visualized by 131I- or 99mTc scintigraphy. In our consecutive series of such patients (n = 6), both primary tumors and metastases were well demonstrated by ^{111}In-DTPA-D-Phe^1-octreotide. Patient B had a Hürthle cell adenoma in a nodular goitre, which illustrates a differential diagnostic problem. The Ti/B values of the goitre tissue approached that of the adenoma and was much higher than those seen in normal lymph nodes (Figure 2). The Ti/B values for the goitre tissue was much higher than that seen for normal thyroid tissue in patient C, also injected five days before surgery. The high uptake of ^{111}In into the goitre tissue may represent cellular uptake not only into thyroid follicular cells, but also into lymphocytes infiltrating the sclerotic parts of the gland. A similar uptake of ^{111}In in the thyroid in benign conditions (endemic goitre, thyroid autonomy and Graves' disease) has been reported previously [27]. The clinical management of this patient was not influenced by preoperative imaging studies.

There are only preliminary observations available on imaging by ^{111}In-DTPA-D-Phe^1-octreotide in patients with thyroid carcinoma. Patient C with papillary thyroid cancer displayed intense uptake in the thyroid tumors (Figure 3). No lymph node metastases were visualized. The T/B values of tumor tissue were three to five times higher than the Ti/B values of normal thyroid tissue. However, the T/B values of macroscopic and microscopic lymph node metastases did not differ from the tumor-free nodes. The clinical management of this patient was not influenced by preoperative imaging.

Benign and malignant thyroid tumors and colloid goitre had increased uptakes of ^{111}In as indicated by ^{111}In-octreotide scintigraphy and by increased T/B ratios. Therefore, ^{111}In-octreotide scintigraphy will probably be of limited value in diagnosing primary thyroid tumors. In that respect, ^{111}In-octreotide scintigraphy will have a similar role as radioiodine scintigraphy, i.e., they are most important after thyroidectomy to diagnose residual neck tumor and distant metastases. Octreotide scintigraphy has three evident advantages:

1. Thyroid substitution therapy does not have to be withdrawn before scintigraphy.
2. Metastases from Hürthle cell cancer and MTC can be visualized (i.e. tumor types that do not concentrate radioiodine) [10, 11, 28, 29].
3. It has a complementary value to radioiodine scintigraphy in diagnosing metastases from papillary and follicular thyroid cancer [26].

The future role of radiation therapy via sstr in patients with thyroid cancer and disseminated disease remains to be investigated.

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