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NUCLEAR MEDICINE IMAGING OF LUNG CANCER AND ESOPHAGUS CANCER

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To

My my lovely wife Gudrun,

our children David and Alice

and mother-in-law Barbro

ABSTRACT

Background: Somatostatin receptors (SSTRs) occur in cancer tissue, and ^{99m}Tc-depreotide is a labelled somatostatin receptor analogue, binding to SSTRs subtype 2, 3, and 5.

Purpose: The general aim of the present thesis was to study somatostatin receptor scintigraphy (SSTRS) with ^{99m}Tc-depreotide in the diagnosis and characterization of cancers in the lung and oesophagus.

Study I evaluated the diagnostic value of the SSTRS with ^{99m}Tc-depreotide in 99 patients with suspected lung cancer. The sensitivity to detect malignancy was 94%, and to detect lung cancer 98%. The specificity was calculated on two sets of data. When all cases are used, the specificity was 52%. If the 12 pneumonias are excluded, the specificity was 77%.
Study II was performed on 19 patients with histologically proven non-small-cell lung cancer (NSCLC), where the expression of SSTR subtype 2 was looked for and found by immunochemical methods. The quantitative evaluation of ^{99m}Tc-depreotide was performed using region-of-interest analysis and includes tumour counts/cm^3, background counts/cm^3, and the ratio between tumour and background counts. SSTR subtype 2 expression was positively correlated to the degree of the tumour's differentiation ($p < 0.05$). ^{99m}Tc-depreotide uptake in tumour cells did not correlate with tumour grade or SSTR subtype 2, MIB-1, or p53 expression.
Study III showed the feasibility of imaging oesophageal carcinoma with SSTRS with ^{99m}Tc-depreotide and optimal time intervals for imaging. None of the 13 cancer-free Barrett's oesophagus patients in this study showed an increased ^{99m}Tc-depreotide uptake.
Study IV investigated the expression of SSTRs of subtype 2A, 2B, 3, and 5 in 28 patients with suspected oesophageal cancer, where expression was detected in small amount in adenocarcinoma and was absent in squamous cell carcinoma. There was no correlation between the ^{99m}Tc-depreotide uptake and the amount of SSTRs, and no correlation between the amount of SSTRs and the differentiation grade of the tumour.

Conclusion: SSTRS with the labeled somatostatin receptor analogue ^{99m}Tc-depreotide has a very high sensitivity for detecting lung cancer. A negative scintigraphy strongly suggests a benign lesion, and the method is useful in decision making with respect to surgery.

There is an expression of SSTRS subtype 2 in NSCLC with a positive correlation between tumour differentiation and presence of SSTR subtype 2. There is no correlation between ^{99m}Tc-depreotide uptake compared to tumour differentiation, presence of SSTR subtype 2, p53, or MIB-1, and SSTRS cannot be used as a prognostic factor in patients with lung cancer.

SSTRS with ^{99m}Tc-depreotide of oesophageal cancer is feasible, but not suitable, for either screening or primary diagnosis, because of the method's modest sensitivity. However, this method has a high specificity. The majority of patients with adenocancer of the oesophagus have a low amount of SSTRs, while most of the patients with squamous cell cancer do not have any of SSTRs.

Keywords: Lung cancer; oesophageal cancer; Barrett's oesophagus; ^{99m}Tc-depreotide scintigraphy; prognostic factor; somatostatin receptor expression; immunohisto-chemistry.

LIST OF PUBLICATIONS

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IV. **Quantitative assesment of ^{99m}Tc-depreotide uptake in esophageal cancer and precursor conditions and its reflection in immunohistochemically detected somatostatin receptors.**
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Manuscript

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LIST OF ABBREVIATIONS

Ac	Adenocarcinoma
CT	Computed Tomography
^{64}Cu	Radioactive nuclide of Copper
DAB	Diaminobenzidine
D-cells	Cells that contain somatostatin receptors
DOTA	1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid
DTPA	Diethylenetriaminopentaacetic acid
EUS	Endoscopic ultrasound
18F, ^{18}F	Radioactive nuclide of Fluor
FDG PET	18Fluoro-deoxy-glucose positron emission tomography
^{68}Ga	Radioactive nuclide of Gallium
^{68}Ga-DOTA-NOC	Radiolabelled DOTA -NI,NII,NIII,NIIII-tetraacetic acid-1-Nal3-octreotide
^{68}Ga-DOTA-TATE	^{68}Ga-DOTA-D-Phe1-Tyr3-Thr8-octreotide or ^{68}Ga-DOTA-D-Phe1-Tyr3-octreotate
^{68}Ga-DOTA-TOC	Radiolabelled DOTA-Phe1-Tyr3-octreotide
GH	Growth hormone
HOSEM	OSEM in Hermes
HRCT	High Resolution Computed Tomography
^{111}In	Radioactive nuclide of Indium
Ki-67	An antigen and a marker for cell proliferation.
^{177}Lu	Radioactive nuclide of Lutetium
MAPK	Mitogen-activated protein kinase
MBq Milli Becquerel	Used in the International System of Units (SI), the becquerel (Bq) is the unit of radioactivity. One Bq is 1 disintegration per second (dps).
MIB-1	Molecular Immunology Borstel, the antibody against Ki-67
MRT	Magnetic Resonance tomography
NET	Neuroendocrine tumour

NP	Neuropeptide
NSCLC	Non-small-cell lung cancer
OSEM	Ordered subset expectation maximization
P53	A nuclear phosphoprotein whose function is classified as a tumour suppressor gene
PRRT	Peptide receptor radionuclide therapy
ROI	Region of interest
SCLC	Small-cell lung cancer
SPECT	Single Photon Emission Computed Tomography
SPN	Solitary pulmonary nodule
SqCC	Squamous cell cancer
SST	Somatostatin
SSTR	Somatostatin receptor
SSTR1, SSTR2A, SSTR2B, SSTR3 and SSTR5	Somatostatin receptors of 5 different types: 1, 2A, 2B, 3, and 5
SUV	Standardized uptake value
TNM staging	Staging of tumour spread. T is local spread of the tumour mass, N is spread to lymph nodes, and M is distant metastases
US	Ultrasound
VOI	Volume of interest
^{90}Y	Radioactive nuclide of Yttrium

1 INTRODUCTION

1.1 BACKGROUND

Cancer arises through a variety of mechanisms resulting in uncontrolled cell division, which play an important role in tumour development and spread. By defining key pathways in those proliferative processes, the ambition has been to make it possible to target specific metabolic pathways' or receptors' steps, allowing tumour detection and collection of prognostic information relevant to diagnosis as well as treatment. Selective receptor-targeting radiopeptides have emerged as an important class of radiopharmaceuticals for molecular imaging and therapy of tumours that overexpress peptide receptors on the cell membrane. After such peptides labelled with gamma-emitting radionuclides bind to their receptors, they allow clinicians to visualize receptor-expressing tumours non-invasively. Peptides labelled with beta-particle emitters could also eradicate receptor-expressing tumours. For evaluation of tumour receptor expression and to increase the value of cancer targeting using radiopeptides, researchers have introduced and evaluated different radiolabelled analogues of peptide families, such as somatostatin, cholecystokinin, gastrin, bombesin, substance P, vasoactive intestinal peptide, and neuropeptide analogues. The somatostatin receptors (SSTRs), which are overexpressed in a majority of neuroendocrine tumours, represent the first and best example of targets for radiopeptide-based imaging and radionuclide therapy.

Somatostatin (SST) is a polypeptid which is primarily produced in the hypothalamus and pancreas and normally occurs in the nervous tissue, hypothalamo-pituitary system, and peripheral tissue like that of the gastro-intestinal canal, endocrine pancreas, kidneys, spleen, prostatic gland, and thyroid gland (1, 2). Two molecular forms of somatostatin, SST-14 and SST-28, have been identified (3, 4). In mammals these two peptides are encoded by a single gene that yields a peptide (preprosomatostatin) that is subsequently cleaved to generate SST-14 or SST-28. It is a modulator of neurotransmission, cell secretion, and cell proliferation (5). SST is also considered to be a neurotransmitter, a neurohormone, or a local hormone acting via autocrine or paracrine mechanisms (5). It is also an inhibitor of secretion of various hormones like neuroendocrine hormones, growth hormone, glucagone, insulin, and gastrin (6). Somatostatin and somatostatin analogues inhibit tumour growth (7, 8). SST has an inhibitory action through the mitogen-activated protein kinase (MAPK) pathways. This could be one effect of somatostatin signalling (9). MAPK activation is important for cell proliferation (10); therefore inhibition of MAPK is likely to contribute to the antiproliferative effect of SST. The role of SST in stimulating apoptotic mechanisms in SST2- or SST3-expressing cells (11, 12) is another notable antiproliferative mechanism.

SST could have two different actions against tumours, partly by direct mechanism such as the above mentioned, or by indirect mechanism, for example, by octreotide-induced inhibition of pituitary growth hormone (GH) secretion, which reduces growth on GH-dependent tumours (13). Another indirect mechanism action against tumours could be SST analogues acting by inhibition of angiogenesis (14, 15).

Somatostatin receptors (SSTRs) The SST acts by binding on protein-coupled receptors called somatostatin receptors (SSTRs). There are 6 subtypes of SSTRs: SSTR1, SSTR2A, SSTR2B, SSTR3, SSTR4, and SSTR5 (2, 5, 16). These 6 subtypes are encoded by different genes, except SSTR2A (5). SSTRs occur in some tumours in much higher concentrations than in normal tissue. High concentrations of SSTRs have been found, for example, in neuroendocrine tumours (17–19), gastroentero-pancreatic tumours (20, 21), phaechromocytomas (22, 23) and to a lesser extent small-cell lung cancers (24). Other tumours that express SSTRs are lymphomas (25), renal cell cancers (26), mesenchymal tumours (27), gastric tumours (28, 29), and hepatocellular tumours (30, 31).

There are 3 main ways to identify SSTRs in normal tissues and in cancer cells. One is by autoradiography with receptor binding with radioactive labelled SST analogues, different substances binding to the different somatostatin receptors SSTR1, SSTR2, SSTR3, SSTR4, and SSTR5 (32, 33). The second method is with receptor mRNA; human tumours often express multiple SSTRs subtype mRNAs, as reported first in pituitary adenomas (34, 35, 32) and gastroenteropancreatic tumours (32). The third method is receptor immunohistochemistry. This method has the advantage of a high cellular resolution. But the results depend on the quality, selectivity, and specificity of the applied antibodies. Even highly specific antibodies SSTR2A such as R2-88 can weakly cross-react with unrelated proteins (36).

1.2 SOMATOSTATIN RECEPTOR SCINTIGRAPHY (SSTRS)

The first radiolabelled SST analogue used for diagnostic purpose was an iodinated ^{125}I- or ^{123}I-Tyr3-octreotide (36). By linking chelators DTPA (diethylenetriamino-pentaacetic acid) or DOTA (1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid), which are indispensable molecules, to accept certain types of metallic radio-isotopes (37), this also improved the biodistribution profile with a shift from a gastrointestinal excretion pathway to a predominant renal excretion (36).

Octreoscan (^{111}In-DTPA-D-Phe1octreotide) is the most widely used radiopharma-ceutical for SSTRS. The best and most consistent results are found in tumours expressing a high density of SSTRs, namely the majority of neuroendocrine tumours, but also meningiomas and medulloblastomas (38, 39). Successful scintigraphy has also been reported for other tumour types, with lower or non homogeneous SSTR density, such as breast cancer, lymphomas, or renal cell carcinomas (40–44).

Depreotide (P829) is an SST analogue, which is a cyclic synthetic peptide that can be labelled with Technetium-99m, yielding ^{99m}Tc-depreotide. It is known under the commercial names NeoTect (USA) and NeoSpect (Europe) (45, 36). Depreotide binds to SSTR2A, SSTR2B, SSTR3, and SSTR5, while Octreoscan binds to SSTR1, SSTR3, and SSTR5. There are some advantages ^{99m}Tc-depreotide scintigraphy over ^{111}In-Octreoscan: besides the fact that ^{99m}Tc-depreotide has high affinity to the SSTR2 receptor, which occurs in many tumours, ^{99m}Tc-depreotide has a shorter half-life, allowing the patient to be imaged the same day, and a lower radiation dose compared to ^{111}In-Octreoscan. The disadvantage of imaging with ^{99m}Tc-depreotide is the relatively high abdominal background and the impossibility of performing delayed imaging due to the short half-life of the tracer, thus making it less suitable for the detection of abdominal tumours. ^{99m}Tc-depreotide was extensively applied to imaging of solitary pulmonary noduli (46–48).

Tracers for Positron Emission Tomography (PET) The PET camera allows a higher spatial resolution compared to the gamma camera, which makes it possible to image tumours with an even higher accuracy. In fact, PET imaging of neuroendocrine tumours (NET) is a rapidly evolving field, closely connected to the development of novel radiopharmaceuticals. NET can be easily visualized on PET scans using an array of receptor-based tracers. More recently the labelling of SST analogues with ^{68}Ga, ^{64}Cu, or ^{18}F resulted in the development of specific PET tracers (DOTA-peptides) that are currently employed in clinical trials in NET patients (^{68}Ga-DOTA-TOC, ^{68}Ga-DOTA-NOC, ^{68}Ga-DOTA-TATE) with very promising results, reported to be superior to other imaging modalities (computed tomography (CT), magnetic resonance tomography (MRT),or SSTRS (49–55).

1.3 THERAPEUTIC USE OF SST

Peptide receptor radionuclide therapy (PRRT) with radiolabelled SST analogues is a promising treatment option for patients with inoperable or metastatic neuroendocrine tumours. Symptomatic improvement may occur with all of the various ^{111}In, ^{90}Y, or ^{177}Lu–labelled SST analogues that have been used. Since tumour size reduction was seldom achieved with ^{111}Indium-labelled SST analogues, radiolabelled SST analogues with beta-emitting isotopes like ^{90}Y and ^{177}Lu were developed. Reported anti-tumour effects of [^{90}Y-DOTA(0),Tyr(3)]octreotide vary considerably between various studies: Tumour regression of 50% or more was achieved in 9 to 33% (mean 22%) of patients. With [^{177}Lu-DOTA(0),Tyr(3)]octreotate treatments, tumour regression of 50% or more was achieved in 28% of patients and tumour regression of 25 to 50% in 19% of patients; stable disease was demonstrated in 35% and progressive disease in 18%. Predictive factors for tumour remission were high tumour uptake on SSTRS and limited amount of liver metastases. The side effects of PRRT are few and mostly mild, certainly when using renal protective agents. Serious side effects like myelodysplastic syndrome or renal failure are rare. The median duration

of the therapy response for [^{90}Y-DOTA(0),Tyr(3)]octreotide and [^{177} Lu-DOTA(0), Tyr(3)]octreotate is 30 months and more than 36 months, respectively. Lastly, quality of life improves significantly after treatment with [^{177}Lu-DOTA(0),Tyr(3)]octreotate. These data compare favourably with the limited number of alternative treatment approaches, like chemotherapy (36, 56–64).

Another possibility for tumour treatment could be the use of unlabelled somatostatin coupled to cytotoxic agents. One of the most efficient compounds is AN-238, a potent cytotoxic radical 2-pyrrolinodoxorubicin linked to the somatostatin octapeptide RC-121, (36, 65). However AN-238 has not yet been tested in clinical trials (36).

Apart from patient selection for radionuclide therapy, other imaging applications of SSTRS include localization of primary tumours, detection of metastatic disease (staging/restaging), dosimetry (prediction of response and radiotoxicity), monitoring effects of surgery, radio(nuclide)therapy or chemotherapy, and detection of progression of disease or relapse (follow-up).

1.4 LUNG CANCER

Lung cancer is the second most common cancer in both men and women in the Nordic countries combined, with rates only superseded by prostate and breast cancer, respectively, while it is the most common cause of cancer death. The incidence of lung cancer per year in Sweden has increased since 1958, and during 1990–1994 was slightly over 2800 (66). While the incidence rates in males have been decreasing in Sweden since 1980, the incidence rates in females have increased. The mortality trends closely follow those of incidence, reflecting the very unfavourable survival of the patients in general (67).

Histopathologically, there are two main groups of lung tumours: small-cell lung cancer (SCLC) and non-small-cell lung cancer (NSCLC). There are several subgroups of tumours among the NSCLC, with adenocarcinoma (Ac) the most common, followed by squamous cell cancer (SqCLC). Other forms of cancer in the lungs are bronchioloalveolar carcinoma and adenosquamous lung cancer, mucoepidermoid, and large-cell lung cancer.

The 5-year survival rates are closely related to the disease stage at the time of diagnosis, which is illustrated in Table 1 (68).

Table 1. Comparison of overall survival between TNM classifications by using the sixth edition of TNM classifications.

Sixth edition TNM	Deaths/no.	Median survival (mo)	5-Year survival (%)	P-value
T1	120/830	53	78.7	–
T2	166/525	46	60.9	<.0001
T3	58/98	35	34.5	<.0001
T4	38/79	33	36.9	.8373
N0	192/1148	52	77.0	–
N1	60/161	45	54.2	<.0001
N2	122/212	32	28.4	<.0001
N3	8/11	11	0.0	.0002
M0	360/1497	50	68.2	–
M1	22/35	24	27.1	<.0001
Total	382/1532	49	67.3	–

P-value: Significance value from log-rank test of survival hazard functions relative to preceding row.

Improved radiologic imaging techniques have affected the clinical staging of lung tumours. Recent recommendations for revisions to the TNM classification and staging system for non-small-cell lung carcinoma attempt to incorporate these new data, with the intent of refining clinical groups to lead to more appropriate therapy for individual patients. Also, improvements in radiologic detection techniques have led to the discovery of smaller lesions, which may be successfully treated with less rigorous chemotherapeutic and surgical protocols.

The radiological diagnostic procedures routinely applied in lung cancer diagnosis include plain X-ray of the lungs and computed tomography of the thorax and abdomen for diagnosing spread of the tumour. The most common manifestation of lung cancer is a solitary pulmonary nodule (SPN) smaller than 3 cm in diameter, which is usually found during CT, or a solitary pulmonary mass larger than 3 cm in diameter. Diagnostic evaluation of focal pulmonary lesions should be accurate and efficient to facilitate prompt resection of malignant tumours, when possible, but surgery should be avoided in cases of benign disease.

The systematic literature review showed that dynamic CT with nodule enhancement yielded the promising sensitivity for identifying a malignant SPN (98 to 100%) with

less favourable and often poor specificity varying between 54 and 93%. In studies of CT-guided needle biopsy, non-diagnostic results were seen approximately 20% of the time, but sensitivity and specificity were excellent when biopsy yielded a specific benign or malignant result (69).

MRT could be used in special situations to see the growth of the tumour to surrounding tissue, for example, overgrowth of Pancoast tumours to the spine with symptoms of nerve root compression or compression on the spinal cord.

To investigate tumour spread, other diagnostic procedures could be used, such as ultrasonography of the abdomen or endoscopic examination of the oesophagus (oesophagoscopy) combined with endoscopic ultrasonography (EUS) and combined with needle biopsy and CT of the brain or bone scintigraphy.

For staging purposes positron emission tomography with F18-Fluoro-deoxy-glucose (FDG-PET) is now a routine method (70–72). Imaging with FDG-PET is a sensitive method to detect pulmonary cancer, but as with CT, it has a limited specificity (71–72). FDG-PET reflects the metabolic activity with glucose turnover and is not specific for the malignancies. Search for other, more tumour-specific imaging agents remains desirable.

SSTRS with ^{99m}Tc-depreotide in patients with lung cancer

A noninvasive functional imaging method based on the principle that malignant nodules have a higher level of somatostatin-avid receptors than benign nodules do was tested in several studies.

^{99m}Tc-depreotide was extensively applied for imaging of solitary pulmonary noduli during last decade. This SST analogue is labelled with gamma-emitting ^{99m}Tc and thus could be easily imaged with a single-photon gamma camera, which is more widely available than PET/CT scanners.

Single-site studies have reported promising results regarding diagnostic performance of ^{99m}Tc-depreotide (47, 73). And two large multicentre studies were conducted. In a United States multicentre study of 114 individuals, Blum et al. (46) reported that ^{99m}Tc-depreotide scintigraphy correctly identified 85 of 88 lung cancers, resulting in 96.6% sensitivity. Methods specificity, however, was lower (73.1%), with false-positive uptake in granulomas and one hamartoma.

In the European multicentre study of 118 patients (48) ^{99m}Tc-depreotide was positive in 65 of 73 patients with a malignant lesion and negative in 30 of 45 patients with a benign lesion, resulting in a sensitivity, specificity, and diagnostic accuracy of 89,67 and 81%, respectively.

^{99m}Tc-depreotide vs. FDG-PET in patients with lung cancer

In the beginning of this century PET technology was available in only few large clinical centres in the United States and Europe, and it was of great importance to compare these two methods. The diagnostic performance between FDG-PET and ^{99m}Tc-depreotide-SPECT in the European multicentre study appears comparable; and scintigraphy with ^{99m}Tc-depreotide is advantageous, since it can be performed with traditional nuclear medicine equipment (48). The results of examinations performed with ^{99m}Tc-depreotide suggest that this test has sensitivity and specificity similar to those of FDG-PET (72).

In a study by Kahn et al. (71) 157 subjects with lung lesions were examined with both methods, and the sensitivities and specificities for detecting malignant disease of FDG-PET were 96% and 71%, and of ^{99m}Tc-depreotide were 94% and 51%, respectively. In the 139 subjects with available complete staging data, FDG-PET correctly staged 76 of 139 patients (55%), and ^{99m}Tc-depreotide correctly staged 63 of 139 patients (45%). The authors concluded that the sensitivity for detection of lung cancer in the primary lesion is equally high for FDG-PET and ^{99m}Tc-depreotide, but the specificity is superior for FDG-PET. The staging accuracy of FDG-PET and ^{99m}Tc-depreotide is similar, but when read with the chest CT, neither scintigraphic examination is sufficiently accurate to stage patients with non-small-cell lung cancer.
Performing a meta-analysis to estimate the diagnostic accuracy of dynamic contrast enhanced CT and magnetic resonance imaging, FDG-PET, and technetium ^{99m}Tc-depreotide single photon emission computed tomography (SPECT) for evaluation of solitary pulmonary nodules (SPNs), Croning et al. (74) found that all these non-invasive methods are accurate in distinguishing malignant from benign SPNs, and differences among these tests are non-significant.

The previous studies with ^{99m}Tc-depreotide in lung cancer patients from our group were in line with research published worldwide and showed promising results in the detection of primary lung cancer and regional lymph nodes (71–72, 75). However, the data on clinical application of this method are missing.

Prognosis in patients with NSCLC

The prognosis for patients with non-small-cell lung cancer depends on a number of factors, including the extent of disease, performance status, weight loss, and TNM staging. Unfortunately, these variables do not always provide a satisfactory explanation for differences in survival rates. Different biological markers may provide further explanations for differences in prognosis. Such biological markers could be p53 or Ki-67 with the corresponding antibody MIB-1.
Prognostic biological markers:

P53 is a nuclear phosphoprotein whose function is classified as a tumour suppressor gene (76–83). Mutations in the p53 gene are currently regarded as the most common genetic alteration in human cancer, and forty different mutations have been identified (78). Mutations in the p53 gene are in some studies correlated to shortened survival in patients with cancer (79, 80), while other studies have shown a better prognosis (81, 82). Still other studies have not showed such correlation (78, 83).

Ki-67 is an antigen that is a large protein and a marker for cell proliferation, that is, correlated to the proportion of cells in the S-phase in the cell proliferation cycle. The S-phase signifies the cells in mitosis, where the DNA in the cell nucleus is replicating. Molecular Immunology Borstel (MIB-1) is the antibody against Ki-67 (84, 85). A majority of studies have shown a correlation between a higher expression of Ki-67 antigen and a worse prognosis in cancer patients (84, 85).

1.5 CANCER OF THE OESOPHAGUS

Cancer of the oesophagus is rare, representing only 0.8% of all new cases of cancer in Sweden 2004 (male: 1.1% and female: 0.4%) (86). Squamous cell carcinoma has long been the most common histological form of cancer in the oesophagus. For the past decade reports have shown a rising incidence of oesophageal adenocarcinoma in most of the western world. The incidence in Danish males has doubled since 1980, with more modest increases observed in the remaining Nordic countries. Among women, incidence has increased in Denmark, but remained stable in Sweden and Norway and declined in Iceland. The trends for mortality closely followed those of incidence. The age-standardized 5-year relative survival was at a low level throughout the period 1964–2003, although it increased somewhat in more recent periods, particularly for Swedish men, where it reached 11% in 1999–2003, and for Swedish women, 14% (87).

Esophageal cancer is an aggressive disease with surgery as an only chance for cure. The TNM classification system is traditionally used to stage oesophageal carcinoma. T1a lesions have less chance of nodal spread, with most series showing an incidence of nodal metastases of <10%, while about 30% of T1b lesions will have nodal metastases. In addition, the number of lymph nodes involved, histology, degree of differentiation, and location seem to have an impact on survival of patients with oesophageal cancer (88). Overall, more than 50% of patients have unresectable or metastatic disease at the time of presentation. The American Cancer Society's Cancer 2009 statistics state that the 5-year survival rate for all patients with oesophageal cancer is only 17%, with better survival for local (33.7%) or regional (16.9%) compared to distant (2.9%) disease at presentation (89).

B**arrett's oesophagus (BE)**

BE is characterized by replacement of squamous epithelium of distal oesophagus with specialized columnar epithelium with goblet cells, thought to be caused by damage from chronic acid exposure, or reflux oesophagitis. Oesophageal adenocarcinoma (Ac) develops in approximately 0.5% of patients with BE per year, and has a poor outcome unless diagnosed early. However, in 10–30% of patients with Ac, BE is not found. It is now generally accepted that Barrett's epithelium can progress through a metaplasia-dysplasia-carcinoma progression but the natural history of dysplasia in Barrett's oesophagus is not well defined. Identification of high-grade dysplasia (HGD) has been considered an indication for oesophagectomy or aggressive endoscopic treatment, since occult invasive cancer has frequently been identified at the time of resection. Without treatment, invasive cancer develops within 3 years in up to half of patients with HGD (90).

The current clinical management of Barrett's oesophagus is hampered by the lack of accurate predictors of progression. In addition, when patients develop cancer, the current staging modalities are limited to stratifying patients into different prognostic groups in order to guide the optimal therapy for an individual patient. Biomarkers have the potential to improve radically the clinical management of patients with Barrett's oesophagus and oesophageal adenocarcinoma, but have not yet entered mainstream clinical practice. This is in contrast to other cancers like breast and prostate for which biomarkers are utilized routinely to inform clinical decisions.

DIAGNOSIS OF OESOPHAGEAL CANCER

Upper endoscopy is the gold standard for the diagnosis of oesophageal carcinoma. While the presence of a mass or a nodule is diagnosed via an upper endoscopy (and presence of cancer proven by biopsy), the depth of the tumour and lymph node involvement cannot be assessed with this modality.

Computed Tomography. The sensitivity and specificity of CT in diagnosing locoregional nodal involvement are 84% and 67%, respectively. For distant organ metastases, the sensitivity is 81% and the specificity is 82% (91). CT has the limitation of diagnosing overgrowth of the tumour to adjacent organs, detecting small tumours of <1 cm and growth to different layers in the oesophageal wall.

FDG-PET. This method has recently been introduced into oesophageal cancer staging and is more accurate than conventional CT imaging, particularly in the detection of distant metastases, and the combination of ^{18}F-FDG-PET/CT is more accurate than CT alone with respect in diagnosing lymph node metastasis close to the tumour, and therefore staging (92). Despite the high sensitivity in detecting oesophageal cancer with ^{18}F -FDG-PET/CT (92), these methods lack specificity; an

elevated ^{18}F-FDG uptake could be seen in different non-malignant conditions, such as inflammation and Barrett's oesophagus (46, 47, 71, 72, 93).

A systematic review has shown a moderate sensitivity and specificity of 51% and 84%, respectively, for the detection of locoregional lymph node metastases, and a sensitivity and specificity of 67% and 97%, respectively, for detection of distant metastases (94).

Endoscopic ultrasound (EUS) is the most accurate non-invasive test for locoregional staging of oesophageal cancer (T and N classification), though distinguishing between early lesions (T1a and T1b) remains problematic. The overall accuracy of EUS for T classification is 84% (94). Rounded, sharply demarcated, homogeneous, and hypoechoic features of a lymph node on EUS indicate malignancy. The overall accuracy of EUS staging of locoregional nodal disease is 77%. The addition of fine needle aspiration (FNA) to EUS further refines the staging of nodal disease, bringing the accuracy up to 85%, (95). EUS is superior to FDG-PET/CT with respect to diagnosing the tumour growth through different layers in the oesophageal wall, overgrowth to adjacent organs, and lymph node metastasis close to the tumour (92, 96–98).

Minimally invasive staging. The use of minimally invasive staging (laparoscopy or thoracoscopy) is not widely practised, because of the improving accuracies of noninvasive methods. Staging laparoscopy can be performed prior to performing a minimally invasive oesophagectomy or definitive resection. Laparoscopy is useful for detecting and confirming nodal involvement and distant metastatic disease that potentially would alter treatment and prognosis in patients with oesophageal cancer. Laparoscopy was reported to change the planned therapeutic approach in 10–17% of patients (99).

SSTRS with ^{99m}Tc-depreotide in patients with oesophageal cancer

We have previously used scintigraphy with ^{99m}Tc-depreotide for the diagnosis of lung cancer, and showed an accurate discrimination between benign and malignant lesions with conventional gamma cameras (46–48). There was a physiologically low ^{99m}Tc-depreotide uptake in the thorax region. Therefore, visualization of overexpression of SSTRs in cancer types other than lung cancer should, theoretically, be feasible. Moreover, our previous results showed that this tracer accumulates both in squamous cancers and in adenocarcinomas (11), which is of clinical relevance in view of the almost exponential increase in the incidence of adenocarcinoma in the distal oesophagus. As of today there are no studies with SSTRS in patients with oesophageal carcinoma.

2 AIMS OF THE STUDY

To establish the sensitivity and specificity of the somatostatin receptor scintigraphy with ^{99m}Tc-depreotide in patients with suspected lung cancer and to determine in which clinical settings it would be beneficial to use this method.

To investigate whether there is an expression of SSTR2 in NSCLC estimated *in vitro* and to determine whether *in vivo* estimated ^{99m}Tc-depreotide uptake reflects the in vitro expression of SSTR2 in NSCLC. Also, to determine whether there is a correlation between ^{99m}Tc-depreotide uptake and the following prognostic factors in patients with NSCLC: tumour grade, presence of MIB-1, and p53.

To evaluate whether oesophageal cancer could be detected scintigraphically with ^{99m}Tc-depreotide and to determine the optimal imaging settings. Also, to investigate the uptake characteristics of ^{99m}Tc-depreotide of the main two cancer types of the oesophagus and relate that to patients with Barrett's oesophagus.

To investigate whether there is an expression of SSTR2A, SSTR2B, SSTR3, and SSTR5 in oesophageal cancer estimated *in vitro* and to determine whether *in vivo* estimated ^{99m}Tc-depreotide uptake reflects the in vitro expression of SSTR2A, SSTR2B, SSTR3, and SSTR5 in oesophageal cancer.

3 MATERIAL AND METHODS

The Ethics Committee at Stockholm Region and the Radiation Protection Committee at the Karolinska University Hospital, Huddinge, approved all studies.

3.1 SUBJECTS

3.1.1 Study I and Study II, lung cancer patients

All patients referred to the Department of Respiratory Medicine and Allergy at Karolinska University Hospital, Huddinge, with suspected lung cancer between April 2002 and January 2004 were asked to participate in the study. After informed consent, 128 consecutive patients were included. No confirmation of pathology was obtained in one patient and because of loss of follow-up he was subsequently excluded. Among the 127 patients, 28 patients were included in a pilot study from April 2002 to March 2003. The remaining 99 patients were evaluated in **Study 1**. There were 47 women and 52 men with a median age of 66 years (range between 31 to 86 years). Sixty-seven patients were smokers, 18 were ex-smokers who had quit for more than one year, and 14 had never smoked.

Among these 127 patients, 19 patients with non-small-cell lung cancer were operated on and tissues samples from the tumours were analysed in vitro with reference to histopathological classification according to the World Health Organisation. Besides standard staining, an immunohistochemical evaluation was performed and these patients are included in **Study II**.

3.1.2 Study III and Study IV, oesophagus cancer patients

Thirty-four patients suffering from dysphagia were referred to the Department of Surgical Gastroenterology at Karolinska University Hospital, Huddinge, and further examined with gastroscopy, EUS, and CT. Of these, 9 were females and 25 males with a median age of 63.5 years (range from 33 to 85 years). Among the 34 patients, 21 had cancer of the oesophagus and 13 had Barrett's oesophagus. The cancer diagnosis was established by histopathological examination of biopsy specimens in 19 cases, and with EUS and cytological confirmation of diagnosis in 2 cases. All patients with Barrett's oesophagus had the diagnosis made at endoscopy and subsequent multiple biopsies.

All specimens were classified as well differentiated (grade = G1), moderately differentiated (G2), or poorly differentiated (G3).

3.2 METHODS

3.2.1 Scintigraphy

For the somatostatin receptor scintigraphy, ^{99m}Tc-depreotide (NeoSpect, GE Biosciences, Sweden) was prepared in accordance with the manufacturer's instructions, and 740MBq ^{99m}Tc-depreotide was injected into an antecubital vein. For Study I and Study II an examination was performed with a double-headed gamma camera (Sopha Medical Vision Scandinavia AB, DST-XL, General Electric, Milwaukee, WI, USA) equipped with low-energy, ultra-high resolution, parallel-hole collimators. For Study I all patients were examined with whole-body scanning and SPECT of the thorax within 2 to 4 hours after injection. The patient's arms were elevated during SPECT and placed alongside his or her body during whole-body scanning. SPECT was done at 64 angles using a 128 × 128 matrix with 40 seconds per angle. Transverse slices were calculated with an iterative algorithm (HOSEM iterative program; Hermes/NUD; Stockholm, Sweden) into a 128 × 128 matrix without attenuation correction. Images were post-filtered with 3D Fourier filter (Butterworth filter with a cut-off frequency of 0.65 cycles/cm order 5.00). In addition to the transversal images, coronal and sagittal 3 mm images of the thorax were reconstructed.

At the time of Study III and Study IV new data were published suggesting a double-phase procedure with SSTRS as increasing methods specificity (47). For that reason, Study III and Study IV SPECT was performed as a double-phase procedure with imaging of the thorax at 2 and 4 hours after injection. Three different gamma cameras were used. Most of the patients (25 of 34) in Study III and (20 of 28) in Study IV were examined with a double-headed gamma camera (E-Cam, Siemens, Erlangen, Germany) and low-energy, high-resolution parallel-hole collimators, using a 128 × 128 matrix, 64 projections through 360º rotation, and an acquisition time of 40 s per projection. An additional 5 patients in both Studies III and IV were examined with a double-headed gamma camera (DST-XL; Sopha Medical Vision Scandinavia AB, Gif-sur-Yvette, France) and low-energy, ultra-high-resolution, parallel-hole collimators, using the same acquisition parameters as above. Finally, 4 patients (in Study III) and 3 patients (in Study IV) were examined with a three-headed gamma camera (Picker IRIX, Cleveland, OH, USA) and low-energy, high-resolution, parallel-hole collimators, using a 128 ×128 matrix, 60 projections through 360º rotations, and an acquisition time of 64 s per projection. Transverse slices were reconstructed with an iterative algorithm (HOSEM v 3.5 iterative program; Hermes/NUD, Stockholm, Sweden) and formatted as a 128 × 128 matrix without attenuation correction. Images were post-filtered with a three-dimensional Fourier filter (Butterworth filter) with a cut-off frequency of 1.1 cycles/cm (order 5.00).

3.2.1.1 Evaluation of scintigraphic images

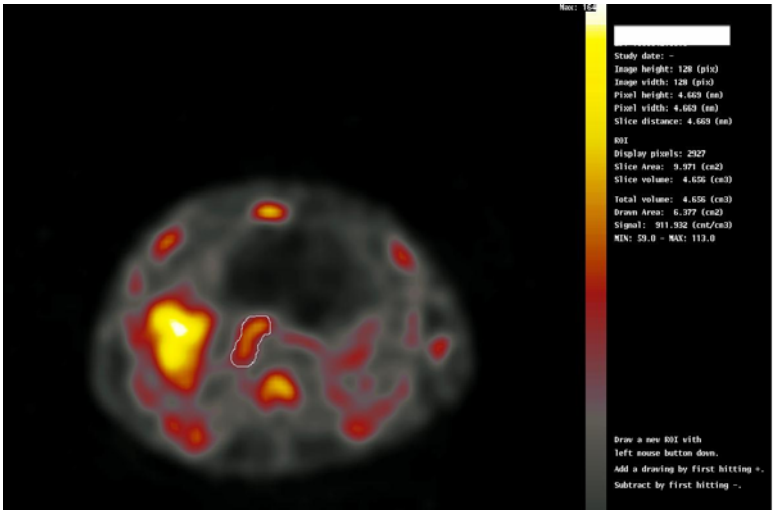
The scintigraphic images were evaluated both through visual assessment (Study I) and through quantitative calculations (in Studies II–IV). Two experienced radiologists, one of whom is also a nuclear medicine specialist, evaluated the scintigrams. The readers were not told of the final diagnosis but were given the radiological findings. The scintigrams were evaluated along with CT, since the reading of the scintigrams requires anatomical landmarks given at the CT investigation (100).

Any visually observed focal accumulation of the ^{99m}Tc-depreotide in the lung parenchyma compared to that of a normal lung was considered as a pathological uptake in patients with suspected lung cancer (Study I and II).

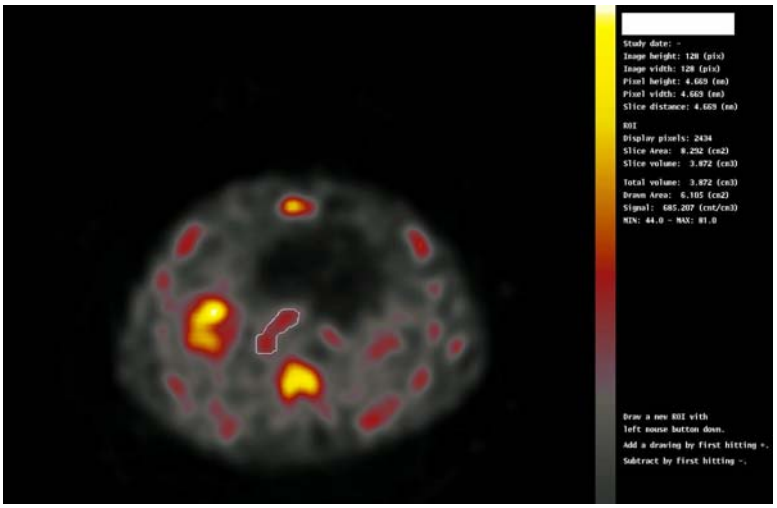
Focal accumulation of the ^{99m}Tc-depreotide in locations other than the lung parenchyma was also registered both with regard to the whole-body images and to the transversal, coronal and sagittal images of the thorax (Study I).

For Studies III and IV any focal ^{99m}Tc-depreotide uptake in the region of the known oesophageal lesion was considered pathological.

The quantitative measurements of the ^{99m}Tc-depreotide uptake (Study II–IV) were made on the SPECT transverse slice images with Hermes computer system by manually drawing a region of interest (ROI) around the lung tumour (Study II) or around the oesophageal tumour (Study III and IV) and by drawing one ROI with the average uptake on the patient's lung parenchyma for background subtraction. The lung parenchyma was chosen as a reference region, as it is known that a normal lung parenchyma as well as brain and muscles show very low expression of SSTRs (101). A volume of interest (VOI) was obtained by adding all ROIs. In-house software, originally developed for volumetric measurements in magnetic resonance images and implemented on a Hermes workstation (Hermes Medical Solution AB, Stockholm, Sweden), was used to calculate the total counts and volume of the tumour and background VOIs, thus giving a count density [counts/cm^3]. To produce a normalized tumour uptake, each patient was normalized to his or her own normal lung parenchyma using the formula $U = (T - B)/B$, where U is the normalized uptake, T is the count density in the tumour, and B is the count density in the lung parenchyma. For patients with negative uptake (i.e. tumour count density lower than the lung background count density), the uptake was scored as zero (Study II–IV).



a)



b)

Figure 1. Evaluation of ^{99m}Tc-depreotide uptake in oesophageal carcinoma 2 (a) and 4 (b) hours after injection.

To increase accuracy and to investigate the intra-observer variability (**Study III** and **IV**), evaluations were performed twice, 6 months apart, by the same radiologist, and the mean value of the two uptake values was used in further analysis. In addition, a second radiologist made individual evaluations in order to investigate the inter-observer variability of the uptake values seen in images taken at 2 hours.

3.2.2 Computed tomography (CT)

CT of the thorax and upper abdomen was performed in all patients in this thesis as a part of routine examination. For Studies I and II all lung lesions (127 patients) were detected by CT, performed between April 2002 and January 2004. The median interval between CT and scintigraphy in Studies I and II was 4 four weeks.

The CT investigations were done both at our hospital (n = 79) and at external departments (n = 20). At our hospital, 46 scans were performed with the multi-slice spiral CT Somatom Volume Zoom (Siemens, Erlanger, Germany), slice thickness 4 × 1 mm, pitch 1.5, and 33 scans with the spiral CT single-slice Tomoscan AVE1 (Philips Medical Systems, Einthoven, the Netherlands) slice thickness 5 mm, pitch 1.5. Images were reconstructed with 5 mm slices in the mediastinum and 10 mm in the lung from both CTs. Contrast media (120 mL) was injected intravenously, and all scans were done while the patients were holding their breath. The CT technology at the external hospitals was essentially the same as at our department.

The CT/chest X-rays were evaluated by two experienced radiologists according to established criteria such as size, calcium and fat content, and the shape and borders of the pulmonary lesions. The age of the patient, smoking habits, and growth rate typical of cancer, as well as signs of invasiveness or metastatic spread, were noted (102). Based on this analysis the probability of lung cancer was graded on a scale from 1 to 3 (Table 1), where a benign lesion was considered 1, indeterminate was considered 2, and a malignant lesion was considered 3.

Table 2. (Study I) Criteria used for CT grading of the radiological probability of malignancy.

Group	Radiological findings	Radiological diagnosis
1	No solid lesion or infiltration resolved since previous chest radiography/CT.A lesion less than 2 cm in size, fat containing, with popcorn-like calcifications, smooth margin, and a sharply defined edge located in upper lobe. No growth for more than 2 years when compared with previous examinations.	Benign
2	A single solid lesion with or without fat or calcifications, with or without cavitations, located anywhere in the lung. No enlarged lymph nodes, no coexisting lung lesions. No previous examinations exist or no growth during previous 6 month.	Indeterminate
3	A solid lesion more than 2 cm in size, without fat or calcifications, irregular contour as well as speculated margin, cavitations with thick, irregular wall, located anywhere in the lungs. Lesion penetrating surrounding tissues, such as vessels and bone. Enlarged lymph nodes, multiple coexisting lung lesions in both lungs. Lesion growth during last 6 month when compared with previous examinations.	Malignant

3.2.3 PET/CT

Some patients in **Study I** and in **Study III** were investigated with PET/CT at Karolinska University Hospital, Solna using a Biograph 64 TruePoint PET/CT scanner (Siemens Medical Solutions, Erlangen, Germany). All patients fasted for at least 6 h before the examination. After 1 h rest, 4 MBq/kg bodyweight (bw) of [18F]-2-fluoro-2-deoxy-D-glucose (FDG) was administered intravenously. One hour later, a PET/CT examination from the middle skull to the proximal thigh was initiated.

Serum glucose levels were routinely measured to ensure that all patients had a normal serum glucose level at the time of examination (G7.0 mmol/L). Diabetic patients were generally instructed to keep their regular schedule of glucose-controlling drugs. The examination started with a low-dose CT without administration of contrast medium for correction of photon attenuation and scattering. Directly upon this, the PET examination was made using a full-ring dedicated scanner with an axial field-of-view of 21.5 cm. Acquisition was made during 3 min at each bed position during normal tidal breathing. Images were reconstructed with CT-based attenuation and scattering corrections using the iterative ordered subsets expectation maximum reconstruction. A full-dose CT with and/or without administration of intravenous contrast medium was performed directly afterward by a continuous spiral 64-slice technique with a voltage of 120 kV, a pitch of 0.8, a slice thickness of 1.2 mm, and a rotation speed of 0.5 s per revolution. In the acquisitions made for photon attenuation and scattering correction, the current was 50 mA•s, while otherwise the tube current modulation routine was applied. Examination was made while breath holding at a mean inspiratory level. For contrast enhancement, Ioversol (Optiray® 350 mg I/mL, Tyco Healthcare Deutschland GmbH, Neustadt/Donau, Germany) was used. The dose was 1 mL/kg bw. In patients 970 kg bw, 10 mL of contrast medium was added. The injection speed was 1.5–2.5 mL/s.

3.2.4 EUS

EUS was performed by way of gastroscopy with an ultrasound probe. All EUS procedures were accompanied with fine needle biopsy. There are two kinds of electronic echo endoscope, the radial and the linear. The radial gives an image in 360 degrees that is perpendicular to the instrument, while the linear gives an image parallel to the axis of the instrument. Only the linear provides a guidance to biopsy. The frequencies used are higher than for ordinary transabdominal ultrasound; frequencies used are between 5 to 30 MHz. The higher the frequency, the higher the resolution will be with the lower penetration and vice versa. The patient fasts before the examination and the examination takes between 15 and 60 minutes to perform (103).

3.2.5 Immunohistochemistry

For determining the somatostatin receptor 2A (SSTR2A) (**Study II**) we used an antiserum raised from the sequence of the C-terminal portion of this somatostatin receptor (Gramsch Laboratories, Schwabhausen, Germany). For MIB-1 receptor determination the monoclonal Mouse Anti-Human Ki-67 Antigen, clone MIB-1 (DakoCytomation, Glostrup, Denmark) was used. For p-53 receptor determination the monoclonal Mouse Anti-Human p53 Protein, clone DO-7 (DakoCytomation, Glostrup, Denmark) was used. The antibody against SSTS2A was applied to tissue sections at a dilution of 1/20000. The anti-MIB-1 antibody was applied at a dilution of 1/80. The anti-p53 antibody was applied at a dilution of 1/40. The immuno-peroxidase technique using the streptavidin-peroxidase kit (DakoCytomation) was used to reveal the immune reactions.

For MIB-1 and p53, the number of positive nuclei was calculated on 10 randomly chosen areas of the tumour histological sections and the result expressed as percentage.

To evaluate the positivity of SSTR2A, an image analysis method was used. Applying the HIS (hue, saturation, and intensity) model (Gonzales) the saturation of the immunoperoxidase stain was calculated (range 0–1), and SSTR2 expression was graded on a three-point scale, with one representing the lowest expression. To avoid bias due to background influence, the mean value calculated in the normal bronchial epithelium was subtracted from the value of the tumour positive areas. The algorithm was written by one of us (AC).

The procedure was different in **Study IV**. For immunohistochemical assessment of the different SSTRs (2A, 2B, 3, and 5), the Bond system (Vision Bio Systems Ltd. Australian, Melbourne) was used. Tissue specimens of the oesophageal tumours and biopsy material from the patients with Barrett's oesophagus were processed and prepared for immunostaining by use of monoclonal antibodies. The pretreatment to achieve the epitope was performed by heat treatment and with the enzyme pronase. The tissue sample was first treated with peroxidase. The antibody was diluted 1000 times and the enzyme pronase was diluted 50 μL in 7000 μL. The tissue sample for SSTR2A and SSTR2B was pretreated with the diluted enzyme solution and with the enhancer for 10 min. The tissue sample for SSTR3 was pretreated with H1 = Citrate buffer pH = 6, without enzyme and without enhancer for 20 min, and the tissue sample for SSTR5 was pretreated with H2 = EDTA buffer pH = 9 without enzyme and without enhancer for 40 min.

After this pre-treatment the samples were incubated with the antibodies for 30 min and the incubation temperature was between 37 and 100ºC. The development was then performed with diaminobenzidine (DAB) and then stained with hematoxylin.

The Bond Polymer Refine Detection System was a compact polymer system with high sensitivity where peroxide block, intensive DAB dyeing, and hematoxylin contrast dyeing was included. This gave the dyeing high intensity combined with a sharp definition, without the use of streptavidin and biotin. By this, it was excluded that non-specific dyeing occurs because of the presence of endogenous biotin, which occurs in large amounts in some tissues in the gastrointestinal channel. During testing of the antibodies, pancreas and skin were used as a positive control to exclude false positive results. Both positive and negative controls were used at the incubation and dyeing steps.

SSTR2A/SS800 was the antibody against the SSTR2A, SSTR2B/SS860 was the antibody against SSTR2B, SSTR3/SS850 was the antibody against SSTR3, and SSTR5/SS890 was the antibody against SSTR5. The enhancer was a copper intensification. The buffers used were H1 = Citrate buffer pH = 6 and H2 = EDTA buffer pH = 9.

The (SSTR) concentration was graded as no receptor presence = 0, small amounts = 1, moderate amounts = 2, and large amounts = 3.

3.2.6 Statistics

Study I. Sensitivity and specificity of SSTRS with ^{99m}Tc-depreotide in lung cancer diagnosis were evaluated in this study. Sensitivity is the number of true positive test results compared to all positives. Specificity is the number of true negative test results compared to all negatives.

Study II. Extreme values may bias results when only two variables are being examined; relationships between tumour grade, ^{99m}Tc-depreotide uptake, SSTR2 expression, MIB-1, and p53 receptors were therefore analysed using Spearman Rank order correlations. The resulting correlation coefficients with corresponding p-values were calculated, and the correlation coefficient was considered significant if the corresponding p-value was 0.05 or less.

Study III. Due to the small number of patients in each group, a non-parametric test was chosen. A two-sided Mann-Whitney U test was used to investigate the difference in uptake between malign and benign tumours, implemented in Statistica 9.0 (StatSoft Inc., Tulsa, OK, USA). Data were analysed based on both the 2-hour and the 4-hour post-injection recordings. A difference in uptake was considered significant if the p-value was less than 0.05. To assess intraobserver and interobserver variability, intraclass correlation coefficients (ICC) were determined.

Study IV. Because extreme values may bias results when only two variables are being examined, relationships between ^{99m}Tc-depreotide uptake, tumour grade, and amount of the different studied SSTRs were analysed using Spearman rank correlations. Corresponding p-values were calculated and considered significant if the p-value was less than 0.05.

4 RESULTS

The findings from the different studies are summarized below.

4.1 STUDY I

This study was conducted after the results of our pilot study showed the rationale of the use of ^{99m}Tc-depreotide in patients with suspected lung cancer. The sensitivity of SSTRS with ^{99m}Tc-depreotide was 94% to detect malignancy and 98% to detect lung cancer. The specificity was calculated on two sets of data - when all cases are used, the specificity is 52%. If the 12 pneumonias are excluded, the specificity is 77%. The diagnostic accuracy of the method in different settings is presented in Table 3.

Of the total of 66 malignancies, 62 were positive on ^{99m}Tc-depreotide scintigraphy, thus true positive n = 62, yielding a sensitivity of 94%. Of the 58 lung malignancies, 57 were positive on ^{99m}Tc-depreotide scintigraphy, yielding a sensitivity of 98%. Among the other 8 malignancies with origin other than the lungs, 5 were positive on ^{99m}Tc-depreotide scintigraphy: these were 2 lymphomas, 2 malignant melanomas, and 1 breast cancer metastasis.

Only one lung cancer was negative on ^{99m}Tc-depreotide scintigraphy, this one a 1 cm bronchioloalveolar carcinoma situated in the apex of the left upper lobe. Three of the malignancies with origin other than the lungs were negative on ^{99m}Tc-depreotide scintigraphy. These were a rectal cancer metastasis, an adenoid cystic cancer metastasis, and the rib chondrosarcoma case). A chondrosarcoma is not a lung tumour, but it occurred in the thorax and was mistaken for one, which is why it was still retained for the assessment of the method. Thus there were false negative n = 4. There was no focal ^{99m}Tc-depreotide uptake in 17 of 33 patients with benign diseases, true negative n = 17. These were all 10 hamartomas, 1 pneumonia, 1 pleuritis, 1 sarcoidosis, 1 granuloma, 1 granulomatous inflammation, and 2 benign lesions which were probably hamartomas.

There was ^{99m}Tc-depreotide uptake in 11 of the 12 pneumonias, in 1 fibrosis, in 2 round atelectases, 1 pleural fibrosis, and 1 pneumoconiosis. Thus false positive n = 16.

Table 3. Diagnostic accuracy of scintigraphy with ^{99m}Tc-depreotide.

	Whole study population N = 99 in %	**Lung cancer and benign lesions N = 91 in %**	**All lesions except pneumonias N = 87 in %**
Sensitivity	94	98	94
Specificity	52	52	77
Accuracy	80	81	89
NNP	91	94	81
PPV	79	77	93

NPV = negative predictive value. PPV = positive predictive value.

4.1.1 Correlation between CT and scintigraphy with ^{99m}Tc-depreotide

All 51 cases radiologically graded as 'malignant' showed ^{99m}Tc-depreotide uptake (i.e. 50 were true positives and 1 was a false positive). More than 1/3 of all lesions in this study were graded as radiologically 'indeterminate' (n = 39). Among these lesions, 16 were malignant, and ^{99m}Tc-depreotide uptake was found in 12 of them. It was absent in 3 patients with metastatic disease and in a patient with bronchioloalveolar carcinoma. In the group of radiologically 'indeterminate' lesions 23 were benign and ^{99m}Tc-depreotide uptake was absent in 13 of them (i.e. true negatives), while 10 cases showed ^{99m}Tc-depreotide uptake and were classified as false positives. Among these 10 cases, 8 were pneumonias, 1 was pleural fibrosis, and 1 pneumoconiosis. If pneumonias are excluded, the number of false positives falls to two. Of nine 'probably benign' lesions, five (3 pneumonias and 2 round atelectasis) showed ^{99m}Tc-depreotide uptake and were false positives.

4.1.2 Extra pulmonary ^{99m}Tc-depreotide uptake

There was ^{99m}Tc-depreotide uptake in 204 regional lymph node stations, in patients with both malignant and benign lesions with a sensitivity of 99%, and a negative predictive value of 98% in determining metastatic lymph node involvement. These data were evaluated separately and are not included in this thesis (75). There was also a focal uptake in locations other than the lung or the mediastinum in 21cases. Depending on the clinical situation, some of these findings were further evaluated. Four patients with lung cancer showed uptake in supraclavicular lymph nodes, diagnosed by fine-needle aspiration as metastases from adenocarcinoma (n = 2) or/and squamous cell lung cancers (n = 2). Focal uptake in the bones represented morphologically verified metastases (n = 2), large osteophytes (n = 2), or degenerative changes (n = 2) on CT. There was uptake in the thyroid in one case of Hashimoto's thyreoiditis and in another case of well-substituted hypothyreosis. One

case with an adenocarcinoma of the lung had two focal uptakes in the brain, one representing a metastasis and the other a meningioma. In one case of small-cell-lung cancer there were multiple small brain metastases on the CT, but no depreotide uptake was detected in the whole body images. Metastases in the liver, adrenals, and kidneys are difficult to detect because of a physiological high ^{99m}Tc-depreotide uptake in these regions. This was the case in four patients with proven metastasis in the adrenals and one patient with liver metastasis. Neither was a 2 cm metastasis in the pancreas detected. A 4 cm fibroadenoma of the breast had no uptake. As previously described, there was an unspecific ^{99m}Tc-depreotide accumulation in axillary sweat glands, not to be mistaken for metastasis (104).

4.2 STUDY II

Correlation between ^{99m}Tc-depreotide uptake and immunohistochemically analysed surgical specimens for SSTR2, p53, and MIB-1 was performed in 19 patients with non-small-cell lung cancer (Table 4).

The level of SSTR2 expression was positively correlated with the degree of tumour differentiation (i.e. the higher the level of SSTR2 expression, the more G1 tumours or well-differentiated tumours). This correlation was significant ($p < 0.05$), correlation coefficient (CC) = 0.81.There was no correlation between p53 expression and tumour grade and no correlation between p53 expression and ^{99m}Tc-depreotide uptake.

In contrast, MIB-1 expression was negatively correlated with tumour grade, (i.e. the higher the level of MIB-1 expression, the fewer G1 tumours or well-differentiated tumours). This correlation was significant ($p < 0.05$), CC = −0.46. There was also a slight but significant negative correlation between SSTR2 and MIB-1 expression ($p < 0.05$), CC = –0.57).

There was no correlation ($p > 0.05$) between tumour grade, SSTR, MIB-1, or p53 expression, either absolute or relative to background ^{99m}Tc-depreotide uptake in tumour cells.

Table 4. Histological parameters versus ^{99m}Tc-depreotide uptake in 19 patients with non-small-cell lung carcinoma.

Patient number	Diagnosis	Diff. grade	MIB-1	P53	SSTR2	Total uptake (T) Counts/cm3	Background (B) Counts/cm3	T-B/B
1	Ac	1	3	5	3	1,216,309	517,013	1.353
2	Ac	2	8	25	2	553,900	339,950	0.629
3	Ac	1	4	0	3	917,689	484,209	0.895
4	Ac pap	1	3	0	2	935,053	449,801	1.079
5	Ac pap	1	78	0	1	930,637	409,165	1.274
6	Ac	3	92	80	1	871,396	442,596	0.969
7	Ac muc	3	70	42	1	1,752,289	521,677	2.359
8	Ac	2	50	95	2	525,631	249,345	1.108
9	Ac LC	3	63	70	1	645,724	319,381	1.022
10	Ac	1	12	0	2	551,742	381,868	0.445
11	Sq	2	16	84	2	384,114	219,341	0.751
12	Sq	2	75	93	2	796,728	419,504	0.899
13	Ac	2	64	99	2	1,090,793	520,498	1.096
14	Ac	3	6	11	1	1,255,699	245,327	4.118
15	Sq	3	87	0	1	401,167	168,640	1.379
16	Ac LC	3	18	71	1	409,694	314,048	0.305
17	Ac	3	10	0	1	565,961	348,891	0.622
18	Neu	3	70	0	1	561,902	364,039	0.544
19	Ac bac	1	10	0	3	289,120	145,225	0.991

SSTR2 are somatostatin receptors subtype 2, and the amount of these receptors are graded as 0 to 3, where 0 is no presence and 3 is the highest amount. Differentiation grade is differentiation of the tumour, graded from 1 to 3, where 3 is the lowest differentiation, that is, the most malignant tumour. Diagnoses: Ac = adenocarcinoma, acinar type; Ac pap = adenocarcinoma, papillary; Ac muc = adenocarcinoma, mucoepidermoid; Ac LC = adenocarcinoma, large-cell carcinoma; sq = squamous cell carcinoma; neu = neuroendocrine pulmonary carcinoma, mixed type; and Ac bac = adenocarcinoma, non-mucinous bronchioloalveolar carcinoma.

4.3 STUDY III

This study showed that it is feasible to image oesophageal cancer with labelled somatostatin analogue. Among 34 patients with oesophageal lesions, 21 had cancer and 13 were benign. Of these 21 patients, 16 had pathological uptake of ^{99m}Tc-depreotide (true positive 76%) and 5 were negative (false negative 24%) on visual assessment. Six of the 8 patients with SqCC and 5 of the 11 patients with Ac showed a pathological ^{99m}Tc-depreotide uptake. The remaining 5 patients had false negative uptake of ^{99m}Tc-depreotide (Table 5). The sensitivity of ^{99m}Tc-depreotide scintigraphy in the detection of oesophageal cancer was thus 76%.

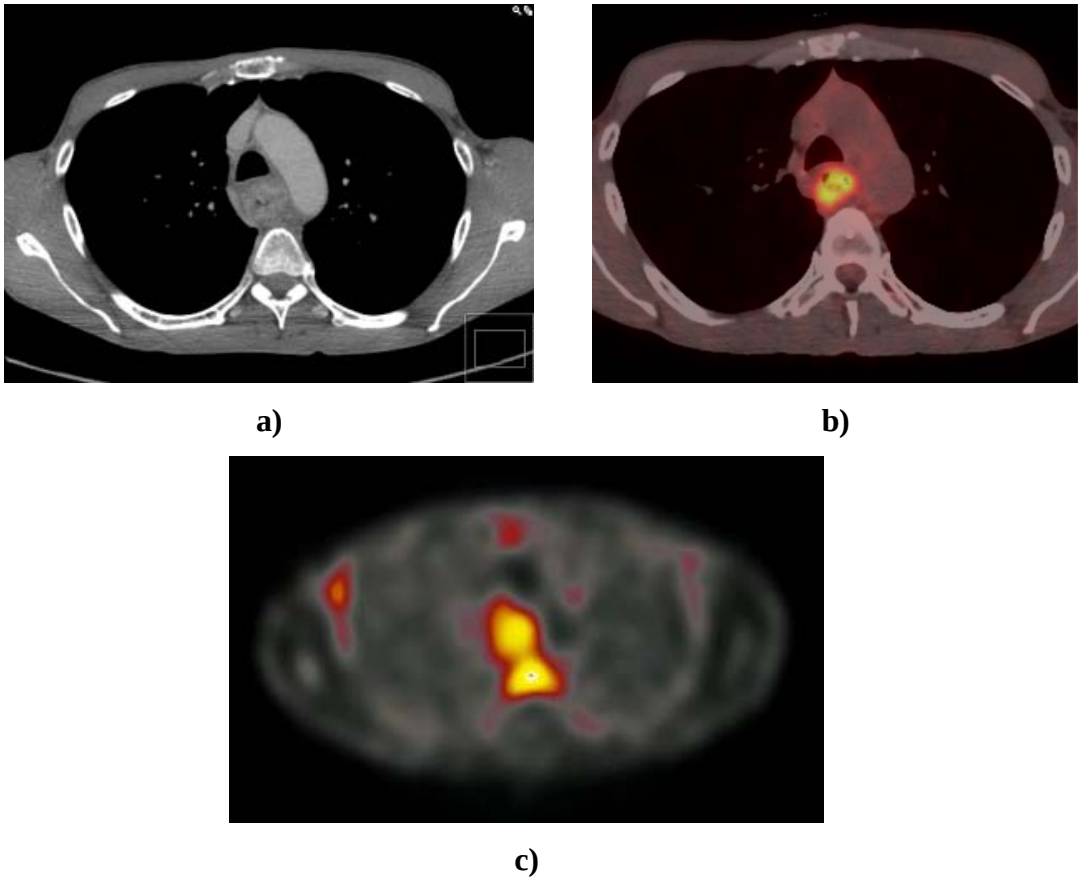


Figure 2. Cancer in the upper part of oesophagus: transversal slice on CT, (a) FDG-PET/CT (b), and ^{99m}Tc-depreotide SPECT (c).

There was no ^{99m}Tc-depreotide uptake in the columnar metaplastic mucosa in any of the 13 Barrett's patients, irrespective of the presence of low- and high-grade dysplasia in the metaplastic epithelium on visual assessment. The specificity of ^{99m}Tc-depreotide scintigraphy in this cohort of patients was thus 100%.

There was no significant difference in uptake of ^{99m}Tc-depreotide between 2 hours and 4 hours ROI delineation and quantitative measurement. A corresponding second ROI delineation and quantification, performed 6 months later, gave consistent results. Both intraobserver and interobserver variability was low, with ICC = 0.97 when comparing the evaluations by the same radiologist (intra-observer), and ICC = 0.96 when comparing the evaluations made by the two radiologists (inter-observer).

A statistically significant difference ($p < 0.005$) was found between ^{99m}Tc-depreotide uptake in malignant lesions compared to that in benign or premalignant lesions (Figure 3), both 2 and 4 hours after injection. The absolute ^{99m}Tc-depreotide uptake value was also higher in all malignant lesions after 2 compared to 4 hours. There was no difference in uptake between Ac and SqCC.

Table 5. Tumour type, size, and location; CT result; and ^{99m}Tc-depreotide uptake in 21 oesophageal cancer patients.

N	Diagnosis	Tumour size in mm	Location	CT	^{99m}Tc-depreotide
1	Sqcc	5	Middle	Neg.	Neg.
2	Ac	17 × 45	Distal	Pos.	Pos.
3	Sqcc	55 × 40	Proximal	Pos.	Pos.
4	Ac in B	65 × 55 × 13	Distal	Pos.	Pos.
5	Ac	90 × 75 × 25	Distal	Pos.	Pos.
6	Imc and B	12 × 9	Distal	Neg.	Neg.
7	Sqcc	30 × 10	Middle	Pos	Pos
8	Ac	20 × 90	Middle	Pos.	Pos.
9	Ac	60 × 25 × 9	Distal	Pos.	Pos.
10	Sqcc	50 × 45	Distal	Pos.	Pos.
11	Small-cell cancer	110 × 24	Middle	Pos.	Pos.
12	Ac in B	60 × 65	Distal	Pos.	Pos.
13	Ac	25 × 15	Distal	Neg.	Pos.
14	Ac in B	20 × 25	Distal	Pos.	Pos.
15	Sqcc	60 × 10	Proximal	Pos.	Pos.
16	Sqcc	15 × 55	Distal	Pos.	Pos.
17	Ac	15 × 50	Distal	Pos.	Pos.
18	Ac	15 × 15	distal	Neg.	Neg.
19	Ac in B	20 × 25	Distal	Neg.	Neg.
20	Sqcc	14 × 5	Middle	Neg.	Pos.
21	Sqcc	23 × 38 × 12	Distal	Pos.	Neg.

Ac = adenocarcinoma, Sqcc = squamous cell carcinoma, B = Barrett's oesophagus, Imc = intramucosal cancer. ^{99m}Tc-depreotide uptake classified as negative or positive based on visual assessment. CT pos = tumour is visible on the CT images. CT neg = tumour is not visible on the CT images.

The absolute ^{99m}Tc-depreotide uptake value was also higher in all malignant lesions after 2 compared to 4 hours. There was no difference in uptake between Ac and SqCC.

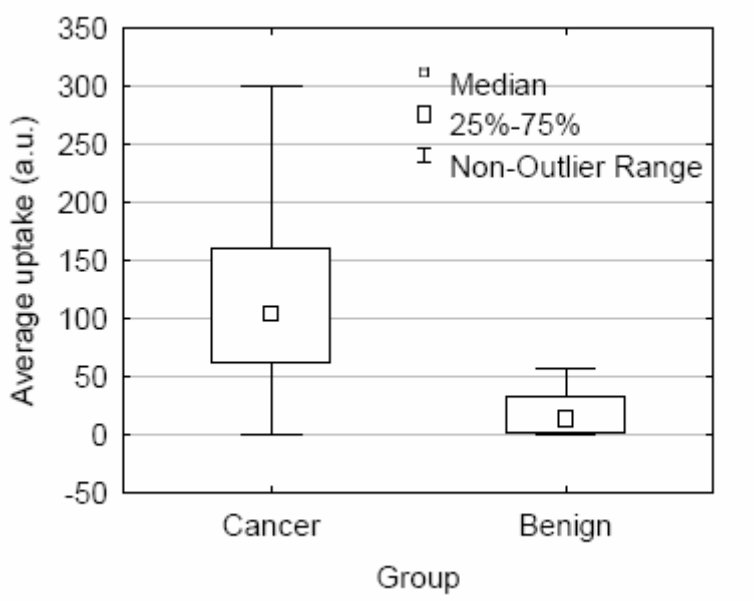


Figure 3 (Study III). ^{99m}Tc-depreotide uptake measured 2 hours after injection in patients with oesophageal cancer and Barrett's oesophagus.

Although this study was not designed to compare the ^{99m}Tc-depreotide scintigraphy with ^{18}F-FDG-PET, we found it of more general interest to make such a comparison. Unfortunately, the number of patients who performed both images was not sufficient for any reliable statistical evaluation, especially of patients without malignancy, and these data were not included in our paper III, but are presented here. Besides, this data would not answer the main question of our study: Is it feasible to detect oesophageal cancer by SSTRS?

Among the 34 patients in this study only 17 were also examined with PET/CT, of whom 15 had cancer and 2 had dysplasia in Barrett's oesophagus without cancer. Among the 15 cancer patients examined with 18F-FDG-PET, uptake was found in 13. False negative results were found in intramucosal cancer in a case of Barrett's oesophagus (12 × 9 mm) and a 5 mm SqCC. Both were missed with SSTRS with ^{99m}Tc-depreotide, as well.

There were 2 discordant results between these tracers, where SSTRS with ^{99m}Tc-depreotide was less sensitive in 2 more cancers, both located in the distal part of the oesophagus: a 23 × 38 × 12 mm SqCC and a 20 × 25 mm Ac (Barrett's).

Table 6. Head-to head comparison of results of SSTRS with ^{99m}Tc-depreotide and FDG-PET/CT.

Diagnosis	Depreotide uptake	FDG PET
Adenocarcinoma Barrett's	+	+
Adenocarcinoma Barrett's	+	+
Adenocarcinoma Barrett's	+	+
Adenocarcinoma Barrett's	-	+
Adenocarcinoma	+	+
Adenocarcinoma	+	+
Adenocarcinoma	+	+
Squamous cell carcinoma	-	-
Squamous cell carcinoma	+	+
Squamous cell carcinoma	+	+
Squamous cell carcinoma	+	+
Squamous cell carcinoma	+	+
Squamous cell carcinoma	-	+
Intramucosal cancer, Barrett's	-	-
Cancer of small cell	+	+
Dysplasia, Barrett's	-	-
Dysplasia, Barrett's	-	-

Our results in the detection of loco-regional lymph node metastases were unsatisfactory. Only 5 of 13 patients with metastases seen with EUS and confirmed by histological examination were clearly detected by ^{99m}Tc-depreotide scintigraphy.

4.4 STUDY IV

One radiologist measured values for ^{99m}Tc-depreotide uptake in April 2009 and October 2009, and a second radiologist measured these uptake values in November 2010. Both intra-observer and inter-observer variability for the quantitative assessment of ^{99m}Tc-depreotide uptake in the oesophageal lesions were low, with the ICC being 0.97 and 0.96, respectively.

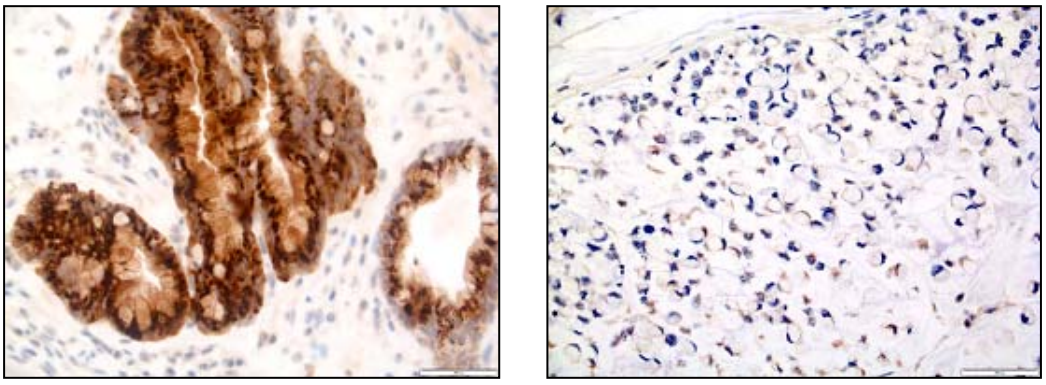
Immunohistochemical detection and semiquantitative assessment of the different SSTRs in 11 patients with Ac are present in Table 7, and the 11 patients with Barrett's oesophagus without cancer in Table 8. Among the 6 patients with SqCC only one patient displayed SSTR5 and the remaining 5 patients were devoid of SSTRs. Among the Ac patients, the majority expressed low amounts of SSTRs; one

patient had none, a few had moderate amounts, and only one patient expressed high amount of SSTR5 (Figure 4).

Table 7. Histopathological and immunohistochemical analyses of adenocancer of the oesophagus.

Patient no.	SSTR2A	SSTR2B	SSTR3	SSTR5	Grade of differentiation	Barrett's
2	1	1	1	1	intermediate	-
4	0	0	0	0	low	yes
5	1	0	1	1	low	-
6	1	1	1	1	high	yes
11	0	0	1	1	low	-
12	1	0	1	1	low	-
13	1	1	1	1	low	yes
14	1	1	1	1	high	-
15	1	1	1	3	low	yes
16	1	2	2	2	high	yes
17	2	2	2	1	intermediate	yes

SSTR expression was graded as none = 0, small amounts = 1, moderate amounts = 2 and large amounts = 3.



a) **b)**

Figure 4. Strongly positive SSTR3 staining in well-differentiated Ac (a); negative SSTR3 staining in poorly differentiated Ac of signet ring cell type (b).

Table 8. Immunohistochemical analyses of 11 Barrett's patients without cancer.

Patient no.	SSTR2A	SSTR2B	SSTR3	SSTR5
7	1	1	3	1
8	1	1	3	-
20	1	1	2	3
21	1	-	1	-
22	1	2	1	2
23	0	0	0	1
24	-	0	0	-
25	1	1	1	2
26	0	0	0	-
27	1	0	1	1
28	2	2	2	2

SSTR expression was graded as none = 0, small amounts = 1, moderate amounts = 2, and large amounts = 3.

No correlation was revealed between the differentiation of the tumour and the expression of different SSTRs, either for the Ac or for the Sqcc. With the exception of a significant ($p \leq 0.05$) correlation ($r = 0.70$) between the presence of SSTR2B and the grading of the Ac, the higher the amount of SSTR2B, the higher the grading of the tumour.

Overall, the observed levels of SSTR2A, SSTR2B, SSTR3, and SSTR5 in Sqcc were significantly lower compared to Ac ($p = 0.001$, $p = 0.019$, $p = 0.0002$, $p = 0.047$, respectively).

The majority of the patients with Barrett's oesophagus expressed SSTRs in their columnar epithelium. The semiquantitative scoring on the abundance of SSTR did not reveal any difference in separation of epithelium between those with dysplastic morphological changes and those without.

No correlation between the ^{99m}Tc-depreotide uptake and the expression of any of the examined SSTRs in the 17 patients with cancer of the oesophagus was found. As well as with lung cancers, there was a tendency for poorly differentiated tumours to have higher ^{99m}Tc-depreotide uptake compared to well-differentiated Ac tumours, but this difference did not reach statistical significance.

The SqCC seemed to express lower ^{99m}Tc-depreotide uptake compared to the adenocarcinoma, but this difference could not be statistically substantiated.
Cases showing positive uptake with the scintigraphic method but negative results in the immunohistological analysis displayed no remarkable degree of inflammation on histopathological examination of the tissue specimens. Neither did we observe an SSRT immunostaining of the inflammatory cells present in the specimens. Not even the non-inflammatory cells (stroma cells, vessels, and others) did so.

Among the patients with Barrett's oesophagus, five had either high or low grade of dysplastic changes in the columnar epithelium. There was a tendency towards higher ^{99m}Tc-depreotide uptake in the epithelium with dysplasia than in that without dysplasia, but this difference could not be statistically verified.

5 DISCUSSION

The main objective of this thesis was to explore the value of somatostatin receptor scintigraphy with ^{99m}Tc-labelled analogue in patients with suspected lung or oesophageal cancer.

5.1 LUNG CANCER

The specificity in diagnosing lesions in the lungs is limited with the currently used methods; therefore surgery is performed on numerous patients with benign lesions. Previous studies with ^{99m}Tc-depreotide have shown promising results in differentiating malignant from benign lesions in the lungs (46, 47, 105, 106). To establish the role of scintigraphy with ^{99m}Tc-depreotide, a selected group of patients with a high probability of lung cancer was studied.

In our **Study I** the *sensitivity* in detecting malignancy was 94%, and in detecting lung cancer, 98%. Thus, all lung cancers but one showed ^{99m}Tc-depreotide uptake. The exception was a 10 mm small bronchioloalveolar carcinoma. A false negative scintigraphic finding can be explained by factors such as size, location, and type of the tumour.

A tumour of this size could be missed, if the difference in uptake between the tumour and the uptake in the surrounding tissue is small, and if the tumour is small the uptake will be diluted in a larger pixel volume, with lowering of the uptake due to partial volume effect. The lower spatial resolution the larger pixel volume. The spatial resolution with the SPECT method is 2 cm. In a European multicentre trial the sensitivity for this method was 93% for lesions >1.5 cm and dropped to 75% when the lesions were ≤1.5 cm (48).

Another reason for false negative results could be the location of the tumour, for example, tumours situated caudally in the lower lobes close to the liver or spleen where the uptake could be hidden by physiological tracer uptake in the liver or spleen (46, 47, 48, 71, 105, 106, 107).

A third possibility for false negative results could be the biological nature of the tumour, that is, the absence of SSTRs. In a study by Martinez et al. (47), the only missed lung carcinoma was of the same size and histological type as in our study, and in a study by Kahn et al. (71) two of the missed tumours were also of the same nature (e.g. bronchioloalveolar carcinoma) and related size.

The *specificity* in our study was in the first calculation rather low, 52%, because of accompanying pneumonia. Therefore, we calculated the specificity after exclusion of the 12 pneumonias, which yielded a specificity of 77%. ^{99m}Tc-depreotide uptake in

inflammatory processes has been described (46, 47, 71, 48, 107) and may be the result of tracer uptake in activated lymphocytes and macrophages (46). Both in infectious and non-infectious granulomatous processes somatostatin receptors have been identified in epithelioid cells. Their presence may cause the false positive ^{99m}Tc-depreotide uptake in round atelectasis (105, 108), granulomas (46, 106), and sarcoidosis (105).

In our study scintigraphy with ^{99m}Tc-depreotide showed a high negative predictive value (94%) for excluding malignancy, which is almost as high as in the study by Rasmussen et al. (107). Our results comport with a study (46) where the sensitivity was 96% and specificity was 73% for malignant lesions in the lungs; it also comports with an FDG-PET study (109) and with a study that compared FDG-PET with ^{99m}Tc-depreotide scintigraphy (72) in a small group of 29 patients. The later study showed a greater sensitivity, but the difference was not statistically significant. Therefore, we concluded that ^{99m}Tc-depreotide scintigraphy is a good diagnostic alternative for centres where PET is not available.

We consider ^{99m}Tc-depreotide scintigraphy as a complementary method in patients with suspected lung carcinoma, applied after routine examinations. Based on the results of our **Study I**, the following strategy for use of ^{99m} Tc-depreotide scintigraphy in the clinical work-up of patients with suspected lung cancer is suggested: If the radiological diagnosis is malignant, ^{99m}Tc-depreotide scintigraphy will not add valuable information. If the radiological diagnosis is indeterminate and pneumonia is excluded, the ^{99m}Tc-depreotide scintigraphy is of value both in malignant and in benign lesions. Because of the high negative predictive value of ^{99m}Tc-depreotide scintigraphy, a negative result will rule out malignancy. This is valuable for avoiding unnecessary surgery. If the radiological diagnosis is benign, ^{99m}Tc-depreotide scintigraphy will not add any new information.

In our study more than one third of the pulmonary lesions were classified as indeterminate after CT examination. This is a great problem in clinical practice and is also reported by Rasmussen et al. (107), using high-resolution CT (HRCT). Rasmussen et al. (107) showed that there is no need for further investigations or follow-up examinations in patients with indeterminate findings on HRCT and negative results in ^{99m}Tc-depreotide scintigraphy.

Even though the study from our group of ^{99m}Tc-depreotide uptake in the regional lymph nodes in patients with lung cancer is not included in this thesis, the results are still important. Absence of ^{9m}Tc-depreotide uptake on scintigraphic imaging can exclude regional lymph node involvement with a high degree of probability and may be useful in clinical practice.

So, lung cancer could be imaged using SSTRS with ^{99m}Tc-depreotide, and this method, by definition, is based on expression of somatostatin receptors (SSTRs) in cancer tissue. Such an expression is a known phenomenon for small-cell lung cancer. The situation with NSCLC at the time we conducted this study was unclear. Despite NSCLC often producing positive results in imaging with ^{99m}Tc-depreotide (48), previous studies (110) have failed to detect the expression of SSTR in NSCLC, while more recently published studies demonstrate an expression (111–113).

Our study showed with immunohistochemical methods that all 19 NSCLC tumours expressed SSTR2. A reason for the contradictory results could be the different analytic methods used by different authors.

In our study, SSTR2 expression correlated significantly with tumour differentiation; the higher the expression of SSTR2, the higher the differentiation of the tumour. However, further studies should be conducted to establish the role of SSTR2 in determining staging and prognosis. This observation might be used for selection of patients for targeted therapy with cytostatic drugs or radio-emitting agents attached to somatostatin receptor analogues.

Surprisingly, no significant correlation between ^{99m}Tc-depreotide uptake determined *in vivo* and SSTR2 expression determined in vitro was found. One explanation may be that SSTRs are also expressed in leukocytes and proliferating neuroendocrine cells around the tumour (114), or in infiltrating lymphocytes (115). Neither lymphocytes, leukocytes, nor proliferating neuroendocrine cells around the tumour were evaluated in this **Study II**. We consequently performed this evaluation in our later study on oesophageal carcinoma. Another explanation may be that ^{99m}Tc-depreotide is known to bind to SSTR subtypes 2, 3, and 5. In **Study II** we measured only SSTR2 immunohistochemically, and even this point we kept in mind in planning our next study on oesophageal carcinoma. We found ^{99m}Tc-depreotide uptake in all tumours, and the uptake in tumours was often higher in G3 tumours compared with G1 tumours, but with some variations, and this correlation were therefore not significant. A study in which semi-quantitative tumour-to-normal lung ratio uptake of ^{99m}Tc-depreotide was compared with different histological types of NSCLC showed that the ratio was highest in SqCC, lower in Ac, and lowest in large-cell carcinoma, but no data about tumour grade were present (116). In our sample 15 tumours are Ac and only 3 are SqCC and 1 neuroendocrine tumour. This may influence the result both for ^{99m}Tc-depreotide uptake and expression of SSTR2 receptors.

Determination of proliferative activity with the monoclonal antibody MIB-1, which binds Ki-67, a nuclear antigen, has been demonstrated to provide prognostic information (84, 85). We did not find a significant correlation between MIB-1 and ^{99m}Tc-depreotide uptake. But we did find a significant negative correlation, that is, the

higher the MIB-1 expression *in vitro*, the lower the differentiation grade of the tumour. We also found a significant negative correlation, that is, the higher the MIB-1, the lower the SSTR2 expression. However, the clinical application of this result is not clear.

The role of p53 is instrumental to the regulation of G1 to S phase transition of the cell cycle. There was no correlation ($p > 0.05$) between p53 and either tumour differentiation or ^{99m}Tc-depreotide uptake. As previously described, some studies found mutations of p53 in more aggressive tumours (77), but others have not found such a correlation (78, 83). Few studies have found a negative prognosis with mutated p53 (79, 80), while other studies have found a better prognosis (81, 82). One study has identified 40 different mutations of p53 (78). In our study, we measured only expression of non-mutant p53, which may explain the lack of correlation. Whether this depends on the type of p53 mutation or is due to factors other than p53 that influence prognosis, such as other genes regulating cell growth or environmental factors, is still unclear.

The results of our studies, however, should be considered in a wider perspective, where PET/CT has an established position in diagnosis in patients with suspected lung cancer. Since the beginning of this century an FDG-PET/CT has been considered to have a major impact on the management of lung cancer patients. Its value has been demonstrated by many publications, meta-analyses, and European/American/Japanese recommendations. Nowadays it is unquestionable that PET combined with CT provides useful information regarding the diagnosis and staging of lung cancer and allows for the delivery of adaptive radiotherapy. In its more common uses, PET/CT has been shown to be cost effective. With the widespread use of new radiotracers, PET/CT will play an increasing role in the evaluation of response to treatment.

During the past two years PET/CT availability in Europe and in Sweden, particularly, is changing, with new scanners installed in several centres. The somatostatin receptor scintigraphy with gamma-photon-emitting isotopes was initially meant for the diagnosis of primary lung cancer. Even if the sensitivity of SSTRS with ^{99m}Tc-depreotide in the diagnosis of primary tumour is close to that of FDG-PET/CT (71, 72), the latter has several advantages, of which the facility for staging is one. The initial cost-benefit of SSTRS is no longer of great value, as it has risen and is now of the same value as FDG-PET.

5.2 OESOPHAGEAL CANCER

As with lung cancer, the specificity in diagnosing lesions in the oesophagus by means of non-invasive radiological methods is limited. Despite the high sensitivity in detecting oesophageal cancer with ^{18}F -FDG-PET/CT (92), these methods lack specificity; an elevated ^{18}F-FDG uptake could be seen in different non-malignant conditions, such as inflammation and Barrett's oesophagus (46, 47, 71, 72, 93).

As SSTRS is based on a mechanism other than FDG-uptake in malignancies, it was of interest to find out whether this method could be useful to differentiate between malignant and benign lesions in the oesophagus. As no one before us had used SSTRS in patients with suspected oesophageal cancer, it was first necessary to determine whether it was feasible. We had a hypothesis that it was feasible to image oesophageal cancer by means of somatostatin receptor scintigraphy with ^{99m}Tc-depreotide. Our hypothesis was based on two facts: first, that the physiological ^{99m}Tc-depreotide uptake in the thorax is low. Therefore, this could be a suitable area for tumour detection in most cases. Second, we hypothesized that oesophageal cancer has the same main histopathological types as lung cancer, such as Ac and SqCC. As scintigraphy with ^{99m}Tc-depreotide is useful for lung cancer detection, this second fact suggested that it could also be applied in oesophageal cancer.

The results of **Study III** support this hypothesis, and imaging of oesophageal carcinoma by means of SSTRS with ^{99m}Tc-depreotide is feasible. The majority of tumours (16 of 21) displayed a significant uptake of the tracer, which could be clearly distinguished from that in the surrounding tissue. It was not unexpected that tumours under or near 10 mm in size were missed in the scintigraphic images. The detection limit of the conventional gamma camera due to poor spatial resolution is well known, and according to widespread consensus, scintigraphic methods are not suitable for screening purposes for any cancer types. Another observation is that even larger tumours in the distal part of the oesophagus, 4 of 13 in the present study, could be missed with this method. Uptake of ^{99m}Tc-depreotide in lung cancers located in the lowest part of the right low lobe (71), and even in oesophageal cancers located at the level of the diaphragm and lower in the abdomen, could be obscured because of the high physiological tracer uptake in the liver.

Our sensitivity figure of 76% is only an approximate value, due to the small number of patients in this study. Still, this is somewhat lower than both the sensitivity for detecting lung cancers (46, 47, 71, 72) with the same tracer and that for detecting lung cancer with FDG-PET (71, 72). While ^{18}F-FDG uptake reflects a metabolic activity of the lesion and is not specific to tumours (71, 72), over-expression of somatostatin receptors on tumour cells could give another valuable piece of information regarding tumour properties.

As a control group, we used patients with Barrett's oesophagus. Barrett's oesophagus refers to an abnormal change (metaplasia) in the cells in the lower end of the oesophagus. It is thought to be caused by damage from chronic acid exposure, or reflux oesophagitis. This metaplasia confers an increased risk of adenocarcinoma. None of the 13 cancer-free Barrett's oesophagus patients in this study showed an increased ^{99m}Tc-depreotide uptake. Meanwhile, only 3 of 5 patients with both cancer and Barrett's oesophagus showed an increased ^{99m}Tc-depreotide uptake, leaving 2 false negative results. The specificity of 100% for the applied method is high, but should be used with caution, as the number of patients was relatively low and the spectra of different benign conditions in the oesophagus were not fully represented in this pilot study.

Our results in the detection of loco-regional lymph node metastases were unsatisfactory. Only 5 of 13 patients with metastases seen with EUS and confirmed by histological examination were clearly detected by ^{99m}Tc-depreotide scintigraphy. This was probably caused by the close location to the primary tumour, where a high depreotide uptake cannot be separated from the uptake in the metastatic lymph nodes. It was disappointing to note that very few of the local, metastatic lymph nodes could be detected by this method. Through this pilot observation it can be envisioned that this technology cannot add to the available methods, such as EUS, in determining the node status in oesophageal cancer during the diagnostic and therapeutic work-up.

As this study is the first of its kind, we considered it important to explore whether the quantitative assessment was reliable between different investigators and over time. Both intraobserver and interobserver variability was very low, meaning that the applied calculations have good reliability.

We applied a somatostatin receptor scintigraphy with ^{99m}Tc-depreotide in a previously non-explored cancer type where the optimal acquisition time was unknown. We used the same starting point for the imaging session as for the standard procedure in the detection of lung cancer, that is, 2 hours after injection (47). During the last decade there has been a trend of performing a double-phase registration in order to increase specificity. The double-phase registration is based on assumptions that the relative tracer uptake in benign lesions decreases with time, while uptake in malignant lesions remains high, or even increases with time (47, 117–120). This approach is now routine for parathyroid scintigraphy, and its use has also been suggested for scintimammography, tumour imaging with FDG-PET, and somatostatin receptor scintigraphy with ^{99m}Tc-depreotide in lung cancer patients (117–120). In order to optimize the imaging procedure from the very beginning, we performed double-phase registrations with imaging 2 and 4 hours after injection. Our results showed that in the majority of patients the absolute uptake decreased more rapidly in the background (i.e. the normal lung parenchyma compared to the malignant lesions

in the oesophagus), resulting in a higher relative uptake in cancer over time. As both the 2-hour and the 4-hour quantitative evaluations showed a statistically significant difference ($p < 0.005$) between ^{99m}Tc-depreotide uptake in malignant lesions and in lesions without cancer, the 4-hour imaging seems unnecessary, and thus could be omitted for practical reasons.

Although future immunohistochemical studies would explore the density, distribution, and localization of somatostatin receptors in the SqCC and Acs, our data from **Study III** indicate that there is no major difference as reflected by the similarity in tracer accumulation between these two major tumour types. It is, however, of particular interest that in patients with Barrett's oesophagus, no accumulation of tracer was observed either in those with or in those without dysplastic histomorphologic changes in the columnar epithelium. Since there is no corresponding pre-neoplastic condition, concerning the SqCC development, it can be hypothesized that the somatostatin receptor expression reaches far higher levels in infiltrative neoplastic growth than in the intraepithelial neoplastic disease states. If so, this observation may be potentially very important and offer unique clinical opportunities, for example, when PET/CT or somatostatin receptor scintigraphy with ^{99m}Tc-depreotide technologies are applied.

What could the future clinical application of our results be? Obviously, the method is not suitable for either screening or primary diagnosis, because of the method's modest sensitivity. Could the uptake of ^{99m}Tc-depreotide be related to the prognosis of the oesophageal tumour?

To answer this question we continued to explore in **Study IV** the tissue correlate to these *in vivo* observations by immunostaining of different somatostatin receptors in the respective tumours, and even in a precancerous condition. One important prerequisite for the potential implementation of the scintigraphy technology was the high level of intra- as well as interobserver agreement in the assessments. However, coming back to the originally formulated issues, the following statements seem to be justified:

(1) SSTRs are expressed in oesophageal carcinoma, and more abundantly so in adenocancer specimens; (2) in vivo ^{99m} Tc-depreotide uptake does not obviously correlate with the immunohistochemical detection of SSTR receptors of different subtypes in oesophageal carcinoma; (3) there is a questionable and clinically irrelevant correlation between the expression of these SSTRs and the grading of adenocancer; and (4) finally, we found that Barrett's columnar epithelium contains these receptors, which can be displayed by ^{99m} Tc-depreotide scintigraphy .

Based on the fact that the columnar epithelium of the stomach harbours substantial amounts of somatostatin cells (D-cells), it did not come as a surprise that we found SSTRs in adenocancers—in Barrett's oesophagus but not in Sqcc. The variability among tumours and patients was unpredictable, and therefore, it can be assumed that our initial theory of introducing the idea that SSTRs are involved in key pathways for the development of these neoplastic processes cannot be supported by the present findings. The robustness and strength of these observations are reinforced by the fact that we carefully investigated many of the other subtypes of SSTR not previously determined for patients with NSCLC, such as SSTR2B, SSTR3, and SSTR5. Moreover, we were unable to find a clear correlation between the SSTR expression and the dysplasia scorings of the Barrett's cases. The present observation that in adenocancers there might be an association between the grading of the tumour and the intensity of the somatostatin receptors to be stained, can either be a finding obtained by chance or be logically based on the reasonable assumption that the more differentiated the tumour is, the closer it resembles the 'normal' columnar epithelium, where the D-cells are quite abundant.

The expression of SSTRs of different subtypes in the presently investigated patients with oesophageal carcinoma did not correlate with the ^{99m}Tc-depreotide uptake on the scintigraphic imaging. This is in accord with our previous study on patients with NSCLC. Attempts have been made to explain and understand why tumours with high uptake of labelled somatostatin receptor analogue ^{99m}Tc-depreotide in scintigraphic images do not regularly express SSTRs on immunohistochemical examination of relevant tissue specimens. Kwekkeboom (114) et al. and Machac et al. (115) have suggested that the ^{99m}Tc-depreotide uptake on scintigraphic images may be due to the presence of accompanying leucocytes or activated neuroendocrine cells around the tumour cells (114) or in the surrounding granulomatous tissue (115). We tried to clarify this option by examining thoroughly the blocks from every one of our patients concerning signs of inflammation and the content of inflammatory cells, stroma cells, and vessels. By doing that, we could not observe any deviation in a direction that could explain the lack of correlation between uptake and the SSTR density. What other explanations for these findings can be considered? Is it possible that ^{99m}Tc-depreotide scintigraphy is more sensitive to detecting SSTRs than the corresponding immunohistochemical methods used? Does ^{99m}Tc-depreotide bind nonspecifically to other structures or receptors on the cell surface than those residing in the D-cells? Is expression of SSTRs a dynamic or stable process, and which of these is picked up by the scintigraphic technology? Many questions remain to be answered before this method could be implemented into clinical practice.

We noted a tendency for poorly differentiated tumours to have higher ^{99m}Tc-depreotide uptake and this could be caused by nonspecific binding to areas of the cell surface, which could be more common on tumour cells with poor differentiation

compared to high differentiation. This corresponds to our previous observations in NSCLC, where poorly differentiated tumours had a higher ^{99m}Tc-depreotide uptake. However, this tendency was not statistically significant, either. In order to explore corresponding relationships in more detail much larger study cohorts are required.

Patients without cancer but with a precancerous condition were enrolled, since it would be of special value to have a tool that could aid in the early detection of those who will subsequently develop neoplasia. Although we found somewhat higher ^{99m}Tc-depreotide uptake in patients with dysplasia compared to those without, the overlap was substantial. Even in the immunohistochemical analysis the tendency was there to show that those with dysplasia more often expressed SSTRs (16 of 17) compared to those without dysplasia (13 of 21). The clinical value of these findings has to be further explored and substantiated in larger patient samples and with longitudinal evaluation.

6 FUTURE ASPECTS

This thesis is based on the use of a single-photon-emitting tracer, but the results should be applicable or even better with positron-emitting tracers. Use of PET/CT cameras combines a better spatial resolution of functional PET imaging with detailed anatomical information, leading to a higher sensitivity. A multitude of new PET analogues are applied, whereas [^{68}Ga-DOTA^{0}, Tyr^{3}] octreotide (DOTATOC) (56), or [^{68}Ga-DOTA^{0}, Tyr^{3}] octreotate DOTATATE (33) are likely to become the new standard for somatostatin receptor imaging with PET. This is because these analogues have a high affinity for the somatostatin receptor subtype 2, and because ^{68}Ga is a generator product with a relatively simple labelling (63). Another somatostatin analogue used for PET is DOTANOC, which has been useful for neuroendocrine tumours. This analogue binds to somatostatin receptors 2, 3, and 5 (51, 52). This analogue is also coupled to ^{90}Y or ^{177}Lu, forming ^{90}Y- or ^{177}Lu-labelled DOTANOC, which is used for peptide receptor radionuclide therapy (PRRT). It seems desirable that peptide used in diagnostic imaging mimics the peptide used later for therapy.

After the successful visualization of somatostatin receptor–positive tumours, a logical next step would be to use radiolabelled somatostatin analogues as a treatment of these patients. Such attempts were undertaken in patients with inoperable and or/metastatic neuroendocrine tumours. While the objective responses for chemotherapy with the median time to progression is reported to be less than 18 months, PRRT with ^{90}Y-octreotide (DOTATOC) or ^{177}Lu-octreotate (DOTATATE) performs considerably better with a median time to progression of 30 and 40 months, respectively (63), and significantly improves quality of life (64).

Are there any questions of clinical use that could be further explored with SSTRS? Among the patients with lung neoplasms, 25% are represented by neuroendocrine tumours, whereas 2% are represented by typical carcinoid and 0.2% by atypical carcinoid (121). About 25% of patients with lung carcinoid are asymptomatic, so that bronchial carcinoids are found incidentally.

When imaged with FDG-PET/CT, these types of malignancies cause diagnostic difficulties. The FDG uptake could be absent, reflecting low metabolism of bronchial carcinoids. Carcinoid in the abdomen, as a subtype of neuroendocrine tumour, is successfully imaged with SSTRS and consequently treated with radiolabelled SSTR analogues. Even though in each published study, concerning imaging with SSRTS of patients with lung cancer, there are only a few carcinoids included, the dedicated studies of carcinoid in the lung are missing and could be conducted in the near future.

Concerning patients with oesophageal lesions, a study of whether uptake of SSTR analogues on scintigraphy could predict the natural history of Barrett's oesophagus and its malignization is obviously the next step in the direction of future research.

7 CONCLUSION

Study I. ^{99m}Tc-depreotide uptake has a very high sensitivity for lung cancer. It also has an acceptable specificity when pneumonias are excluded. Negative scintigraphy strongly suggests a benign lesion. This method is very useful in establishing the benign nature of hamartomas. It can be recommended clinically in decision making with respect to surgery.

Study II. There is an expression of SSTR in NSCLC. The degree of tumour differentiation correlates positively with SSTR2A concentration. No significant correlation between SSTR2 scintigraphy with ^{99m}Tc-depreotide, MIB-1, and p53 expression, and tumour grade was found. We found a significant negative correlation between MIB-1 expression, SSTR2A expression, and tumour grade. No correlation was found between p53 and tumour grade, or ^{99m}Tc-depreotide uptake.

Study III. Scintigraphic examination with ^{99m}Tc-depreotide is feasible for imaging oesophageal cancer, but the method is not suitable either for screening or primary diagnosis, because of the method's modest sensitivity. Our first results showed high specificity, which should be used with caution, as the number of patients was relatively low. Acquisitions starting 2 hours after injection are optimal, and suffice for imaging.

Study IV. The majority of patients with Ac do express a low amount of SSTRs, while those are absent in the majority of patients with Sqcc. There was no correlation either between the ^{99m} Tc-depreotide uptake and the amount of SSTRs or between the amount of SSTRs and differentiation grade of the tumour. There is somewhat higher ^{99m}Tc-depreotide uptake and expression of SSTRs in the immunohistochemical analysis in patients with Barrett's oesophagus and dysplasia compared to those without dysplasia.

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