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# APPLICATION OF NUCLEAR MEDICINE METHODS IN PATIENTS WITH BREAST CANCER

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Painting on cover: "Dance" by Marion Forssell, 2005

***With respect to the gender perspective, I dedicate this work to all patients with breast cancer. Even though this dissertation is based on female patients, the disease can occur in men. Breast cancer accounts for less than 1% of all male cancer and 1% of all breast cancer (Levi F et al). Men should be investigated and treated with the same dignity as women.***

*Levi, F, Lucchini, F, La Vecchia ,*
*Epidemiology of male breast cancer*
*(Editorial) Eur J Prev, vol 11(4) August 2002: 315*

***So little done, so much to do.***
***Cecil John Rhodes (1853-1902)***

# ABSTRACT

While physical examination, mammography and aspiration cytology or core biopsy form the basis in the diagnosis of breast cancer, additional examinations are sometimes required in the evaluation of unclear breast masses. The study deals with the application and evaluation of different nuclear medicine techniques in the diagnosis and treatment of breast cancer.

**Study I** evaluates the addition of mammoscintigraphy with $^{99m}$Tc-MIBI to the standard diagnostic techniques. Adding mammoscintigraphy in 96 women with unclear breast lesions increased the sensitivity of palpable cancers from 96% to 100%, and from 89% to 97% for non-palpable cancers. This, on the other hand, reduced the specificity.

**Study II** It has been reported that $^{99m}$Tc-MDP, an agent for bone scintigraphy, may be used also to depict breast cancers. Combining this would represent a rationalization. Since no robust comparisons are reported, the uptake of $^{99m}$Tc-MDP and $^{99m}$Tc-MIBI was compared in 20 women with large breast cancers. $^{99m}$Tc-MDP depicts the tumour with the same uptake versus the background as $^{99m}$Tc-MIBI in post-menopausal women not on hormonal replacement therapy. Otherwise, $^{99m}$Tc-MIBI gives a higher contrast.

**Study III** evaluates the possibility to assess the effect of neoadjuvant chemotherapy (5-FU, epirubicin and cyclophosphamide) of large breast cancer with mammo-scintigraphy using $^{99m}$Tc-MIBI and SPECT. After finished chemotherapy there was a strong reduction of the tumour uptake, while after one therapy course no effect was detected.

**Study IV** In order to localise the sentinel node(s) at surgery of breast cancer, a gamma camera examination is often made after injection of the radiocolloid. To improve the anatomical orientation by visualizing the soft tissue background at this examination, 25 MBq of pertechnetate was administered i.v. also in 41 consecutive patients. As this activity may obscure the lymph node uptake, the number of visualised nodes was compared with that of 47 patients not given pertechnetate. There was no difference in the number of detected nodes between the patient groups. Hence, administration of pertechnetate does not reduce this detectability, while the orientation is improved.

**Study V** $^{99m}$Tc-HMPAO is an agent for examination the regional brain blood flow. The uptake of $^{99m}$Tc-HMPAO was compared with that of $^{99m}$Tc-MIBI in 21 consecutive women with breast cancer (≥1 cm). In the entire patient group, the sensitivity of the agents was the same, while in the individual patients there was a restricted agreement. This may be used to evaluate tumour characteristics *in vivo* since the uptake mechanism of both agents is involved in resistance to anti-neoplastic drugs.

Nuclear medicine examinations are complementary to other methods for the diagnosis and the evaluation of breast cancer. New nuclear medicine techniques have to be evaluated and used if found valuable, or rejected if they do not add any information.



**Keywords:** breast neoplasm, neoadjuvant chemotherapy, radionuclide investigation, sentinel node, SPECT.

## LIST OF PUBLICATIONS

The thesis is based on the following five original articles, which will be referred to in the text by their roman numerals:

I. **Complementary use of scintimammography with $^{99m}$Tc-MIBI to triple diagnostic procedure in palpable and non-palpable breast lesions.**
Wilczek B, Aspelin P, Boné B, Pegerfalk A, Frisell J, Danielsson R. Acta Radiologica 44 (2003) 288-93.

II. **A comparison of $^{99}$Tc$^{m}$-MDP and $^{99}$Tc$^{m}$-MIBI in the detection of breast cancer.**
Wilczek B, von Schoultz E, Johansson L, Larsson SA, Jacobsson H. Nuclear Medicine Communications 21 (2000) 159-63.

III. **Early assessment of neoadjuvant chemotherapy by FEC-courses of locally advanced breast cancer using $^{99m}$Tc-MIBI.**
Wilczek B, von Schoultz E, Bergh J, Eriksson E, Larsson SA, Jacobsson H. Acta Radiologica 44 (2003) 284-87.

IV. **Sentinel node scintigraphy in breast cancer using a dual tracer technique.**
Wilczek B, Sandelin K, Eriksson S, Larsson SA, Jacobsson H. Nuclear Medicine Communications 25 (2004) 135-38.

V. **$^{99m}$Tc-exametazime as a breast tumour–seeking agent: comparison with $^{99m}$Tc-sestamibi.**
Wilczek B, Svensson L, Danielsson R, Celebiouglu F, Larsson SA, Jacobsson H. The Journal of Nuclear Medicine 45 (2004) 2040-44.

# CONTENTS

# LIST OF ABBREVIATIONS

| | |
|---|---|
| **ATP** | Adenosine triphosphate |
| **CT** | Computer tomography |
| **DD** | Double diagnosis (mammography and fine-needle aspiration cytology) |
| **FDG** | [$^{18}$F]-fluoro deoxyglucose |
| **FEC** | 5-fluorouracil, epirubicin and cyclophosphamide |
| **FNAC** | Fine needle aspiration cytology |
| **GSH** | Glutathione |
| **$^{99m}$Tc-HMDP** | $^{99m}$Tc-hydroxymethylene diphosphonate (oxidronate) |
| **$^{99m}$Tc-HMPAO** | $^{99m}$Tc-d,l-hexamethylpropylene amine oxime ($^{99m}$Tc-exametazime, Ceretec®) |
| **i.v.** | Intravenous |
| **[$^{123}$I]-mIBG** | [$^{123}$I]-metaiodobenzylguanidine |
| **$^{99m}$Tc-MIBI** | $^{99m}$Tc-hexa-2-methoxyisobutylisonitrile ($^{99m}$Tc-sestamibi, Cardiolite®) |
| **$^{99m}$Tc-MDP** | $^{99m}$Tc-methylene diphosphonate |
| **MDR** | Multi-drug resistance |
| **MRI** | Magnetic resonance imaging |
| **MRP** | Multi-drug resistance protein |
| **PET** | Positron emission tomography |
| **Pgp** | P-glycoprotein |
| **rCBF** | Regional cerebral blood flow |
| **RES** | Reticulo-endothelial system |
| **SPECT** | Single photon emission computed tomography |
| **SUV** | Standardized uptake value |
| **TD** | Triple diagnosis (physical examination, mammography and fine-needle aspiration cytology) |
| **US** | Ultrasound |

# 1 INTRODUCTION

## 1.1 BACKGROUND

The annual global incidence of breast cancer is estimated to be about one million. This makes it the second most diagnosed malignancy after lung cancer (Schwartsmann et al). Because of a better long-time survival, however, breast cancer is the most prevalent cancer worldwide. In the year 2000, there were an estimated 3.9 millions women alive who have had breast cancer diagnosed within the past 5 years, compared with 1.4 million survivors, men and women, having had lung cancer (Parkin). In the western world, breast cancer is the most common cancer in females. In Sweden it is responsible for approximately 30% of all malignancies affecting women. The lifetime risk of developing breast cancer is estimated to 1 in 10 (Swedish National Cancer Registry).

During the previous decade, the annual incidence of breast cancer in Sweden has increased in average 1.5%, while breast cancer mortality has remained relatively stable and even showed a trend downwards (Duffy et al). Probable explanations to the better survival are the combined benefits of early detection due to increased women awareness, easily available and reliable diagnostic procedures, the introduction of screening programs and better treatments (Tabar et al 2003). The primary treatment of breast cancer is surgical removal. The overall patient survival may also have been improved by adjuvant chemotherapy and/or endocrine therapy (Collaborative Group on Hormonal Factors in Breast Cancer, Early Breast Cancer Trialists' Collaborative Group 1998a, 1998b). Also radiotherapy with modern strategies after breast conserving surgery may have contributed to this trend (Vinh-Hung et al).

Mammography screening has contributed to the overall breast cancer mortality reduction. Only the policy of offering screening seems to have contributed to reduce the mortality. The option probably increases public awareness, resulting in an earlier reporting of breast symptoms (Duffy et al, Tabar et al 2003). Mammography screening has also been shown to directly reduce mortality in different age groups. In the age group 40-69 years the mortality reduction was 44%. In the age group 40-49 years, the mortality reduction was 40-50% in the two Swedish counties using a screening interval of 18 months (Tabar et al 2001). Even in a considerably older age group (70-74 years) a mortality reduction of 24% has been found (Jonsson et al). The two Swedish counties study (Tabar et al 2001) demonstrated that approximately 18% of the mortality reduction was caused by other factors than screening, e.g. improved treatment and public awareness. This indicates that the reduced mortality in patients of different age groups who were offered screening examination was mainly attributable to the screening programs (Tabar et al 2003). The effect of mammography screening on the reduction of breast cancer mortality has also been shown to persist after long-term follow-up (Nyström et al).

Life-style in the western world, early menarche, late age at first child birth, low parity, high alcohol intake, higher socio-economic status, late menopause and obesity at menopause are factors considered to increase the risk for developing breast malignancy

(Magnusson, Beckmann et al, Cabrera et al). Genetically linked breast cancer has been demonstrated and connected to the gene BRCA1 (Miki et al), p53 on the long and short arms of chromosome 17 (Malkin et al) and to the gene BRCA2 on the long arm of chromosome 13 (Wooster et al). Several other less penetrating mutations are also suspected to be involved in breast cancer development, e.g. ataxia-telangiectasia (Athma et al) and HRAS1 (Krontiris et al). Among mutation carriers, the lifetime risk of developing breast cancer has been reported to be 85% for BRCA1 and BRCA2, and approximately 50% for p53 (Ford et al). However, in most populations these genes demonstrate a low prevalence, <1% (Ford et al). Breast cancer development and outcome are issues of strong public interest. The natural history of breast cancer involves a sequential progression through defined clinical and pathological stages. It starts with epithelial hyperplasia, progresses to carcinoma in situ and thereafter to invasive carcinoma with the risk of metastatic disease (Beckmann et al). Breast cancer is a heterogeneous disease with variable malignant potential and metastatic capacity. A full-blown cancer phenotype includes sustained cell proliferation, disregard of growth and differentiation controlling signals, resistance to aptoptosis, ability to invade surrounding tissue and induction of angiogenesis (Polyak et al). All these functions have implications for diagnosis as well as therapy evaluation, underlining the importance of proper patient selection for different treatment modalities and the need to develop methods for early evaluation of the therapy response.

# 2 DIAGNOSIS OF BREAST CANCER

The assessment of breast cancer is based on a dedicated teamwork by surgeons, oncologists, pathologists, cytologists, and radiologists. Triple diagnosis (TD) is the commonly used diagnostic procedure for the diagnosis of breast tumours in Sweden since more than 20 years. It consists of clinical examination, mammography and fine needle aspiration cytology (FNAC). Frequently, core biopsy is also required. In addition, women should learn and practice self-examination. In cases of symptoms (palpable lump, discomfort, nipple or skin retraction, bloody or serous nipple discharge) clinical breast examination, including palpation of the breasts and regional lymph node stations has to be performed by an experienced surgeon or oncologist. However, lesions located deep in the breast, small lesions and diffusely growing lesions especially in fibroglandular breasts are difficult to palpate. This sometimes requires the use of complementary breast tumour imaging methods.

## 2.1 MAMMOGRAPHY

Mammography is the most reliable, widely available and cheapest imaging method for the evaluation of breast lesions. It has also a highest overall sensitivity than ultrasound in detecting pathological changes (Teh et al 1998, Wilson). The sensitivity for detecting breast cancer ranges from 63-90% (Fletcher et al). The medio-lateral oblique view is the ground for screening. Adding a cranio-caudal view increases the detection rate by a mean of 7% (Feigh). In the clinical setting, medio-lateral or latero-medial views (direct lateral views) are also routinely included. They are obtained 90 degrees to the cranio-caudal projection. If a lesion is located in the medial part of the breast, a latero-medial projection, placing the lesion closer to the film will be chosen. Conversely, if a lesion is located in the lateral part of the breast, a medio-lateral projection is performed. Placing the side of the breast in question closer to the film improves image sharpness. If necessary, so-called rolled views (images taken to exclude superimposition) or spot compression views with or without magnification are also acquired.

In dense breasts (i.e. breasts with much parenchyma and little fat) the interpretation of mammograms is difficult and may negatively affect screening programs (Stallard et al). The increasing use of hormone replacement therapy in post-menopausal women in the western countries tends to enlarge this problem, since such treatment can increase the breast density (Lundström et al, Conner et al). A paradoxical effect may be that while hormone replacement therapy renders mammography interpretation more difficult (Litherland et al), such treatment may also increase the risk of developing breast cancer (Magnusson). However, such a risk appears to diminish with time, and 10 years after cessation of such medication the risk was no longer increased (Magnusson, Collaborative Group on Hormonal Factors in Breast Cancer). No evidence of increased breast cancer risk was observed with regard to the use of oral contraceptives (Magnusson). Interestingly, breast density in itself has also been claimed to be a risk factor for the development of breast cancer (Boyd et al).

Digital imaging techniques have improved in recent years and provide satisfactory image quality while permitting lower radiation doses. Digital mammography decouples image acquisition and display, allowing optimization of both processes. A second opinion can also easily be obtained by transmitting the digital data to another centre. Dual-energy mammography, tomosynthesis, digital subtraction mammography are under development (Pisano et al).

## 2.2 ULTRASOUND (US)

Equipment for ultrasound examination is nowadays present in every radiological unit dedicated to breast diagnosis, and the technique is quite frequently used in the clinical setting. While ultrasound alone is not very useful as a screening tool it is well established as a primary complementary tool to mammography (Skaane). Ultrasound guided needle puncture is a common first choice in guided puncture technique as it is fast, reliable, convenient and associated with a lower morbidity than the X-ray guided stereotaxic procedure (Khattar et al, Parker et al, The et al 1998). In dense breasts, ultrasound is recommended as an additional diagnostic tool (Fung et al, Gordon et al, Kolb et al). After mastectomy, ultrasound allows early detection of local recurrences (Edeiken et al). The indications and efficacy of ultrasound have increased due to advances in digital technology and high frequency probes. Doppler examination can reveal invasive components in changes that are only illustrated as microcalcifications on mammograms (The et al 2000). A new trend in this field is the use of intravenous contrast agents made up by micro-bubbles. These agents seem to be promising for studying the tumour vascularity (Madjar et al, Rizzatto et al, Wilson, Meuwly et al). The ultrasound technique is, however, limited by the fact that it is very operator dependant, and in case of impalpable lesions subtle sonomorphologic changes may be misinterpreted.

## 2.3 MAGNETIC RESONANCE IMAGING (MRI)

Contrast enhanced imaging of the breasts with paramagnetic agents was introduced in the late eighties. It has become a technique in patients with unclear breast changes. In our hospital, a study performed on 250 breast lesions showed a sensitivity of 93% and a specificity of 73% (Boné et al 1996). Heywang in 1993 reported a sensitivity of 99.5%, and Kaiser 97.3% (Kaiser 1992, Kaiser et al 1993). The specificity has been reported to vary between 28% and 97% by different investigators (Fischer et al, Gilles et al 1994a, Harms et al, Heywang 1993, Kaiser 1992). This wide range may be explained by different techniques, selection of patients and interpretation guidelines (Boné 1997). Because of the restricted specificity, MRI should be used only in selected patients as a complementary method to routine investigation. The technique is competing with nuclear medicine imaging in women with a known genetic risk for breast cancer, in patients presenting metastatic axillary lymph nodes of unknown primary malignancy, in the pre-operative evaluation in order to exclude multifocality, in monitoring the effect of chemotherapy and in the post-operative follow-up in scar tissue (Kristoffersen-Wiberg, Heywang 1986a, 1986b, 1989, 1990). A correlation between contrast agent enhancement and tumour invasiveness and malignancy grade has also been established (Boné 1997, Boné et al 1998). MRI of the breast has also shown a tumour size that after

finished chemotherapy correlated with the gross histologic size (Gilles et al 1994b). It has a higher spatial resolution (lesions of 4 mm may be detected) than mammo-scintigraphy (Kristoffersen-Wiberg). MRI provides also dynamic imaging by the use of contrast agent enhancement. Thus, Hulka et al have reported a correlation between the extraction blood flow product and microvascular permeability and neoangiogenic activity. Functional MRI of the breast may be useful in monitoring the tumour response to completion of chemotherapy (Delille et al). It has been proven to be better than clinical examination, mammography and ultrasound in the evaluation of tumour response to chemotherapy. The absence of vascularisation on early scans, however, cannot be interpreted as an accurate sign of total cancer remission (Balu-Maestro et al). MRI guided biopsies are performed in some centers, while biopsy guidance by nuclear medicine techniques outside the sentinel node procedure (se below) is still limited to very few centers.

# 3 NUCLEAR MEDICINE

## 3.1 BACKGROUND

Nuclear medicine is the branch of medicine that employs radioisotopes in the diagnosis and treatment of diseases. Naturally occurring radioactive substances were discovered in 1896 by Becquerel. In 1913 de Hevesy created the 'tracer principle' in biology. This contributed to the understanding of the 'dynamic state of body constituents', i.e. living organisms are in a constant state of chemical flux, characterized by a balance between formation and breakdown of body constituents. These principles are applied to the care of patients in the practices of nuclear medicine. The technology makes it possible to define diseases in terms of physiology and biochemistry rather than in terms of anatomy and histopathology by non-invasive methods.

Among the first organs to be examined by nuclear medicine techniques in patients was the thyroid. Around 1940 it was found that the incorporation of radioactive iodide was increased in hyperthyroidism and reduced in hypothyroidism. As new tracers and instruments were developed, eventually every organ of the body was studied by the tracer principle. Today, nuclear medicine is a recognized medical entity in most countries with emphasis on functional and biochemical changes rather than on structural deviations from normal.

## 3.2 DIAGNOSTIC NUCLEAR MEDICINE

In diagnostic nuclear medicine imaging, the tissue distribution and behaviour of a compound with specific biochemical or physiological properties, and labelled with a gamma emitting radioisotope (together making a *radiopharmaceutical*) is studied. Both the radionuclide and the substance labelled are used in so small amounts that they seldom have any pharmacodynamical effects. Consequently, diagnostic nuclear medicine represents pharmacokinetics. Nuclear medicine (scintigraphic) examinations provide functional information based on the properties of the radiopharmaceutical and the mode of delivery. This is in contrast to radiological examinations, which mainly show anatomy based on physical principles. Provided a radiopharmaceutical has a high affinity to a pathological tissue compared to that of surrounding tissues, also very small lesions, e.g. a tumour, may be visualized. This fact also renders nuclear medicine techniques in principle more sensitive for pathological changes than radiological examinations, which always require a certain volume of a lesion in order to be identified. On the other hand, the spatial resolution of nuclear medicine examinations is much lower than that of radiological examinations, why scintigraphic images are more 'blurred'. Nuclear medicine and radiology both represent 'imaging', but must not be regarded as competing diagnostic techniques. Instead, the different qualities together with the limitations of each of the modalities make them complementary. The combination of nuclear medicine examinations and radiological examinations often provides synergistic information.

Diagnostic nuclear medicine is mainly used for *positive* (hot spot) imaging, i.e. a pathological lesion gives rise to an increased uptake contrasting with surrounding normal background activity. Scattered (secondary) radiation from a hot spot may also contribute to its visualization. For certain clinical questions, examinations based on *negative* (cold spot) imaging are made, i.e. a lesion shows a reduced accumulation compared to a surrounding normal uptake. Negative imaging, however, is non-specific and exhibits a restricted sensitivity. The possibility of detecting cold spots largely depends on the size of the lesion and on the amount of surrounding activity. All examinations in the current studies are based on positive imaging.

A large number of radiophysical, technical and radiopharmaceutical contributions to daily nuclear medicine could be mentioned. A considerable achievement was the gamma camera, or Anger camera (for its inventor, Hal O. Anger). This was described in 1958 (Anger), and is today the instrument regularly used for imaging in nuclear medicine. The gamma camera became commercially available in the mid-sixties and replaced rapidly previous rectilinear scanners because of superior performances. Together with the introduction of technetiated radiopharmaceuticals, and especially such tracers for bone scintigraphy (see below), this constituted the beginning of modern nuclear medicine around 1970. Through constant development the performance of the gamma camera has been steadily improved, and especially the introduction of the computer technology has allowed their versatility to be continuously expanded.

The introduction of a clinical gamma camera tomography system, Single Photon Emission Computed Tomography (SPECT) by Larsson in 1980 represented a significant step forwards in nuclear medicine imaging. Planar examinations, as in conventional radiography, are limited by two-dimensional information and thereby also a low contrast resolution. In addition, since nuclear medicine examinations are based on radiation that is *emitted* from the patient a true representation of the activity in the 'third' dimension (the depth) is not obtained at planar examinations. The emitted radiation is always degraded by attenuation in overlying tissues, this causing an under-representation of activity (information) from more deeply located tissue uptake. The half-value for $^{99m}$Tc in water is 4.8 cm. By rotating one or several gamma camera heads around the patient and applying an image reconstruction technique similar to that used at X-ray computer tomography (CT), SPECT provides tomographic (sectional) images of the activity distribution. Compensation for the attenuation of the photons on their way through the body is often routinely performed, while correction for scattered photons, which is more difficult to make, is usually not made at clinical examinations. Nevertheless, SPECT provides a more correct depiction of the activity distribution and is more adequate for quantitative studies than planar acquisitions.

The application of the PET-technique in clinical nuclear medicine represented another important step forwards. Positron Emission Tomography (PET) uses positron ($\beta^+$) emitting radiopharmaceuticals. At annihilation of the positron with an electron ($\beta^-$), two photons with an angle of $180 \pm 0.25^\circ$ are emitted. By detecting the coinciding photons, a tomographic image of the activity distribution can be constructed without the use of a collimator. The higher detection efficiency of this technique entails a higher image quality compared to the single photon technique. PET also allows absolute quantification of radioactivity concentration with higher accuracy than SPECT.

The PET-technology has been available since more than 25 years. Since the radiopharmaceuticals used for this purpose usually are rather short-lived and their production requires close access to a cyclotron, PET has for many years been restricted to a few centres and mainly used for scientific purposes. During recent years, however, the clinical use of PET in oncology has considerably expanded and today examinations with the 'universal' tumour tracer [$^{18}$F]-fluoro deoxyglucose (FDG) have become a routine procedure at many cancer centres. The recent development of high-performing equipments for combined PET- and CT-examination at the same occasion has contributed to this development. All examinations in the current studies are made using the single photon technique (planar scintigraphy or SPECT).

The radiopharmaceuticals very much represent the potential but also set the limits in nuclear medicine practice. The development of the $^{99}$Mo/$^{99m}$Tc-generator ('cow'), and thereby the direct availability of various radiotracers for different purposes had a great impact on clinical nuclear medicine. The first publication suggesting the use of this generator in health sciences was by Richards in 1960, although the achievement represented a considerable interdisciplinary and interphysician communication. The radionuclide $^{99m}$Tc has photon energy of 140.5 keV, an adequate half-life of 6.01 h, and a very low emission of electrons causing any unnecessary radiation burden.

The development of new radiopharmaceuticals, both for single photon examinations and for PET, is rapid. For hot spot imaging it is important to use a tracer being as sensitive and specific as possible for the clinical question at issue. In order to properly evaluate a finding it is important to understand the uptake mechanism of the tracer used. Despite this fact, the uptake mechanism of some commonly used tracers for hot spot imaging are not known in detail why the interpretation, in addition, has to be based on experience. From a general point of view, specificity rather than sensitivity represents a limitation for several commonly used radiopharmaceuticals for tumour diagnosis.

## 3.3 MAMMOSCINTIGRAPHY

Nuclear medicine was involved in the diagnosis of breast cancer as a complement to existing diagnostic modalities as early as 1946 by using [$^{32}$P]-phosphate and a Geiger-Müller counter (Low-Beer et al). Since that, many radiopharmaceuticals have been evaluated and a few are also clinically applied for this purpose. The major single photon emitting tracers showing uptake in breast tumours are summarized in Table 1. Only a few of these radiopharmaceuticals have been used, or are currently used clinically for breast cancer diagnosis.

Breast cancer uptake after administration of $^{201}$Tl-chloride was described in 1978, and this was the first agent to be used in clinical practice for this purpose (Hisada et al). The tracer was originally developed for myocardial scintigraphy. It is physiologically handled as the potassium ion. Its use in clinical practice is hampered by poor physical characteristics, high radiation doses and logistical problems, why its application for

tumour diagnosis never became widespread. It has mainly been used for this purpose in Japan.

**Table 1.**
**Single photon radiopharmaceuticals showing uptake in breast tumours**

| |
|---|
| Radiolabelled monoclonal antibodies and peptides |
| Different monoclonal antibodies labelled with $^{131}I$, $^{123}I$, $^{111}In$ or $^{99m}Tc$ |
| $^{99m}Tc$-pentadecapeptide α M2 |
| Receptor imaging agents |
| $^{111}In$-pentetreotide (somatostatin receptors) |
| $^{99m}Tc$-depreotide (somatostatin receptors) |
| $^{131}I$-E-17-α-iodovinyl oestradiol (oestrogen receptors) |
| $^{123}I$-16α-oestradiol (oestrogen receptors) |
| Nonspecific uptake mechanisms and perfusion imaging agents |
| [$^{32}P$]-disodium hydrogen phosphate |
| $^{67}Ga$-citrate |
| $^{197}Hg$-chlormerodrin |
| Sodium pertechnetate ($^{99m}TcO_4^-$) |
| $^{99m}Tc$-diethylene triamine penta-acetic acid ($^{99m}Tc$-DTPA) |
| $^{99m}Tc$-pentavalent dimercapto succinic acid [$^{99m}Tc$(V)-DMSA] |
| $^{99m}Tc$-methylene diphosphonate ($^{99m}Tc$-MDP) |
| $^{201}Tl$-chloride |
| $^{99m}Tc$-Sestamibi ($^{99m}Tc$-MIBI) |
| $^{99m}Tc$-Tetrofosmin |
| $^{99m}Tc$-sulphur colloid |
| $^{99m}Tc$-labelled erythrocytes ($^{99m}Tc$-RBC) |
| $^{99m}Tc$-glucoheptonate |

$^{99m}Tc$-MIBI ($^{99m}Tc$-Sestamibi) is a 'modern' technetiated radiopharmaceutical for myocardial scintigraphy. In 1987, an incidental uptake of $^{99m}Tc$-MIBI in a lung metastasis from thyroid carcinoma was described (Mueller at al 1987). In 1989 followed a report describing uptake of the tracer in a series of lung tumours (Hassan et al). Uptake in breast cancer was described in 1992 (Aktolun et al). The lateral prone examination of breasts, which today is the clinically common technique, was described in 1994 (Khalkali et al). Thereafter, a large number of articles has been published on the use of $^{99m}Tc$-MIBI at both planar and SPECT imaging in breast cancer. Today it is the most common single photon tracer for this purpose.

$^{99m}Tc$-Tetrofosmin {$^{99m}Tc$-1,2-bis[bis(2-ethoxyethyl)phosphino]ethane, Myoview™} is also a tracer labelled with $^{99m}Tc$ that was developed for myocardial scintigraphy. The

functional and diagnostic properties are very similar to those of $^{99m}$Tc-MIBI (de Jong et al). Uptake in breast cancer was described in 1996 (Rambaldi et al). This has been followed by several reports on its use for the same purpose. Next to $^{99m}$Tc-MIBI, it is the most common single photon agent for mammoscintigraphy. Preparation of $^{99m}$Tc-Tetrofosmin is simpler than that of $^{99m}$Tc-MIBI since a boiling step is not required.

$^{99m}$Tc-MDP ($^{99m}$Tc-methylene diphosphonate) is the most common radiopharmaceutical for bone scintigraphy. The idea to use a diphosphonate for the detection of breast cancer emanates from 1973 (Berg et al), but first after a report of a large series of examinations in 1995 (Piccolo et al) attention was paid to this use of the 'old' agent. The attractive concept is that examination with $^{99m}$Tc-MDP can be used at the same occasion both for the depiction of a primary breast cancer as well as for examination of the skeleton with regard to metastases.

Clinical mammoscintigraphy is usually made by acquiring lateral prone views. This means that the patient lies prone on a special mattress or on an examination couch with semicircular indentations on each side, allowing the breasts to be pending and minimizing the distance between the breast and camera head. Examination is made with the camera head in vertical position. In order to avoid registration of uptake from the contralateral side, a layer of lead is interposed between the breasts. Clear visualisation of the breast and the ipsilateral axilla is obtained in both lateral projections. An anterior projection is also often performed with the patient in a supine position and the hands placed behind the head. This projection includes both breast and the axillary areas and contributes to the localization of a breast lesion. Injection of $^{99m}$Tc-MIBI is ideally made in a pedal vein since the concentrated radiopharmaceutical tends to adhere to the vessel wall upon injection. This may cause confusing uptake patterns in the axillary and supraclavicular regions.

Because of the radiation dose, availability and the more complex examination procedure mammoscintigraphy cannot be used for screening of asymptomatic individuals or for examination of clinical patients to the same extent as mammography. Nevertheless, it has been proven to be a useful complementary method especially in dense breasts, operated breasts with suspicion of recurrence, breasts with implants, breasts with multi-focal changes and in breasts with architectural distortions or otherwise unclear changes where regular structural examination techniques are insufficient. Data from a multi centre trial involving 673 patients examined with $^{99m}$Tc-MIBI indicated an overall sensitivity of 85% and a specificity of 81%. Non-palpable lesions (387) showed a sensitivity of 55-72% (Waxman). Several other prospective studies have shown an overall sensitivity of $^{99m}$Tc-MIBI in the detection of breast cancer of 85%, a specificity of 89%, and positive and negative predictive values of 89% and 84%, respectively (Taillefer). $^{99m}$Tc-MIBI may accumulate in the desmoplastic or stromal reaction of the tumour as well as in the tumour, why tumours as lobular carcinoma (tumours mostly composed of large islands of fibrous stroma) may be more difficult to visualize (Villanueva-Meyer et al 1996). Small tumours and tumours with low metabolic activity may be overlooked with this method, why it is not recommended for lesions smaller than 1 cm. Other possibilities for non-visualization are the tumour location within the breast, size and density of the breast tissue, distance of the tumour from the camera and soft-tissue attenuation. With currently available

standard gamma cameras no lesion detection less than 5 mm has been reported (Taillefer). The accuracy is higher using SPECT (Tiling et al 1998). Dedicated gamma cameras based on semi-conductive detectors for mammoscintigraphy are being developed, and may increase the diagnostic accuracy (Mueller et al 2003).

# 4 RADIOPHARMACEUTICALS

## 4.1 $^{99M}$TC-MIBI

$^{99m}$Tc-MIBI ($^{99m}$Tc-hexa-2-methoxyisobutylisonitrile, $^{99m}$Tc-sestamibi, Cardiolite®) was developed for myocardial scintigraphy as a modern technetiated alternative to $^{201}$Tl-chloride. In solution it is a monovalent cation complex with a central Tc(I) core octahedrally surrounded by six identical alkylisonitrile groups which are coordinated through isonitrile carbon. The surrounding terminal alkyl groups give the complex a moderate lipophilic property (Chiu et al). The mechanisms of uptake are fairly well studied for myocardial imaging. This involves passive distribution across plasma and mitochondrial membranes. At equilibrium, strong negative transmembrane potentials promote largely irreversible concentration of the agent within the inner matrix of the mitochondria, this depending on the cell metabolism (Chiu et al, Delmon-Moingeon et al). The first pass extraction and the blood-clearance is rapid, why the myocardial uptake largely also reflects the blood flow. The mechanism of increased uptake in tumours is not entirely clear. Undoubtedly, increased blood flow in breast tumours compared to normal breast tissue contributes to increased uptake. The relative importance of cellular retention factors versus delivery (like blood flow) is unknown.

In addition to the myocardium, which accumulates only 1-2% of the administered activity, significant amounts are accumulated in skeletal muscles, liver/bile ducts, spleen, stomach, small intestines, thyroid, salivary glands, choroid plexus and kidneys. The augmented uptake in malignant tumours is believed to be caused by stronger negative mitochondrial and plasma membrane potentials secondary to increased metabolism of the tumour cells (Chiu et al). Indirect mechanisms such as an increase of blood flow and capillary permeability have also been suggested to contribute (Aktolun et al, Kao et al).

Tumour growth and invasion is dependant on angiogenesis (Weidner et al). Cancer cells build their own vascular supply by so-called neoangiogenesis. Early uptake of $^{99m}$Tc-MIBI in both benign and malignant tumours is related to the degree of angiogenesis, percentage of badly formed blood vessels and high mitotic activity (Omar et al). In mouse breast cancer models angiogenesis seems to enhance the accumulation of $^{99m}$Tc-MIBI (Ohira et al). Also in human breast cancer a relation between the uptake of $^{99m}$Tc-MIBI and angiogenesis has been reported (Scopinaro et al).

## 4.2 $^{99M}$TC-HMPAO

$^{99m}$Tc-HMPAO ($^{99m}$Tc-d,l-hexamethylpropylene amine oxime, $^{99m}$Tc-exametazime, Ceretec®) was developed to depict the regional brain blood flow (rCBF). It is clinically mainly used for the differential diagnosis of dementia and to depict epileptic foci. The tracer is a racemic mixture of the oxotechnetium(V) complexes with d-HMPAO and l-HMPAO. The complexes have a square pyramidal structure with the apical

oxotechnetium group surrounded by methyl substituents giving the tracer neutral, liphophilic characteristics and being unstable in aqueous solution (Neirinckx et al). The blood-clearance is rapid, and only about 5% of the administered activity goes to the brain. After crossing the blood-brain barrier, fixation depends on interaction with intracellular glutathione (GSH). The radiopharmaceutical is thereby decomposed into hydrophilic species with a low back-diffusion. The finally trapped tracer in the central nervous system remains very stable (Andersen et al). Glutathione is a tripeptide with a free cysteine sulfhydryl that is present at millimolar concentrations in cells. It is the principal intracellular thiol responsible for protecting the cell from oxidative challenge (Rowell et al). The level of glutathione is known to influence the response to tumour treatment, e.g. depletion of glutathione by buthionine sulfoximine increases radio- and chemosensitivity (Bump et al, Suzukake et al).

The uptake mechanism as well as the distribution of the tracer outside the central nervous system has been poorly studied as the tracer is seldom used for this purpose. It is commonly believed that the uptake mechanism is the same in normal extraneuronal structures as well as in tumours. Uptake in tumours implanted in mice was described in 1987 (Hammersley et al). In 1988 accumulation of $^{99m}$Tc-HMPAO in metastatic thyroid cancer in humans was described (Marienhagen et al). After this followed a number of reports describing occasional uptake in head-and-neck tumours, lung tumours, soft tissue sarcomas, thyroid carcinoma, renal cell cancers and hepatocellular carcinomas. Uptake by human breast tumour cell lines was reported in 1997 (Ballinger et al). There are, however, no large prospective studies of the accuracy of $^{99m}$Tc-HMPAO in the depiction of tumours.

## 4.3 $^{99M}$TC-MDP

$^{99m}$Tc-MDP ($^{99m}$Tc-methylene diphosphonate) is the most commonly used tracer for bone scintigraphy. The first practical agent for this purpose was technetiated polyphosphate described in 1971 (Subramanian and McAfee). Soon, a variety of technetiated bisphosphonates were introduced. Several agents have undergone extensive evaluation, but $^{99m}$Tc-MDP, which was described in 1975, remains the most widely used agent for this purpose (Subramanian et al). A bisphosphonate (or a diphosphonate) is a 'man-made' compound characterized by a –P–C–P– structure. The binding to the bone mineral of bisphosphonates is due to this structure, while an antiresorptive effect on bone tissue is influenced by the structure of the side chains (Fleisch). There is a large number of bisphosphonates used both for technical and medical purposes. They may be used e.g. to keep pipes free from calcifications or in toothpaste to protect from tartar. In clinical medicine, these agents are used to treat malignant hypercalcaemia, soft tissue calcifications, Paget's disease of the bone and Charcot's arthropathy as well as to slow down progression of osteoporosis and destructive skeletal lesions as well as pain in malignant disease. Such compounds tagged with the β-emitting radioisotopes $^{186}$Re and $^{153}$Sm are also used for palliation in bone metastases. In contrast to bone scintigraphy, this represents pharmacodynamical use of bisphosphonates.

The use of bisphosphonates at bone scintigraphy represents pharmacokinetics. Very small amounts of substance are administered, and the dosage is made by the amount of radioactivity (MBq or mCi). The exact mechanism behind the osseous uptake of the tracer is not known. It appears that regional blood flow, osteoblastic activity and extraction efficiency are the major factors that influence uptake. Bone uptake is initially rapid, but it gradually decreases. At one hour after injection the osseous activity remains practically stable after correction for physical decay of the radioisotope. On the other hand, renal excretion of the blood-born activity is a continuous process. Imaging is therefore not made earlier than two hours after administration of the tracer in order to obtain an adequate target-to-background activity ratio.

In areas of osteogenic activity, active crystals of hydroxyapatite with large surface areas appear to be the most suitable sites for chemisorption of the bisphosphonate complex with technetium (Frances et al). Consequently, tracer uptake reflects bone turn-over, why the normal skeleton always is always visualised. Most noxious processes cause an increased uptake (a 'hot spot'). This is an unspecific phenomenon reflecting only the reaction of the normal osseous tissue to most pathological processes. On the other hand, the technique is very sensitive to most diseases and injuries affecting the skeleton, why it has become one of the most frequent examinations in clinical nuclear medicine. Its main application has been to depict bone metastases, but is also used for diagnosis and evaluation many other bone diseases. The uptake mechanism of $^{99m}$Tc-MDP in breast tumours is not known. The tumour uptake, however, reaches a peak at 10-20 min, why registration much earlier than at bone scintigraphy is necessary (Piccolo et al).

## 4.4 RADIOCOLLOID (NANOCOLL®)

Human serum albumin colloid with $^{99m}$Tc (Nanocoll®) is one of several radiolabelled colloids used for depiction of the reticulo-endothelial system (RES). It began with radioactive thorium dioxide in 1941 (Maxfield et al). Thereafter, colloidal $^{198}$Au was used during many years for this purpose. $^{197}$Hg-sulphide colloid has also been tried for lymphoscintigraphy. Technetiated colloids were introduced in 1964 (Harper et al), and technetium has now replaced other radioisotopes for labelling of colloids for diagnostic purposes.

By definition, colloid particles in suspension are small enough not to form a sediment, but sufficiently large to scatter incoming light. The size distribution of the colloid affects its distribution and behaviour in the human body. In medical practice, the term 'colloids' is reserved for particle sizes below 1000 nm. Several different radiocolloids were developed in the early 1970s for liver, spleen and bone marrow scintigraphy. The size distribution of the different commercial radiocolloids is usually vaguely defined. It also seems that with certain types of preparations particle sizes may vary from batch to batch (Keshtgar et al). Nevertheless, the size of the colloidal particles has always been optimized so that, after intravenous injection, the pulmonary capillaries are bypassed and the particles are trapped by the reticulo-endothelial system (RES).

Once labelled, radiocolloids must remain stable and have a known and adequate size distribution. Different particle sizes will influence the methodology and the results.

After interstitial injection, the colloidal particles enter the lymphatics and are transported to the lymph nodes where phagocytosis occur, thereby depicting the lymphatic system (Frier). Smaller particles will migrate more rapidly and require rapid monitoring, while larger particle will migrate more slowly and require later monitoring (Keshtgar et al). There is today no consensus regarding the optimal particle size for the detection of the sentinel node. It may even be that certain colloids are better for some tumours or different anatomical regions. In addition to the size distribution, the number of particles and their charge may be important for their behaviour. When in contact with sera, colloidal particles may also change in size (Keshtgar et al). There is today no commercially available radiocolloid specifically designed for lymphoscintigraphy-/sentinel node scintigraphy. Colloids once developed for liver, spleen and bone marrow scintigraphy are used for this purpose in clinical practice. The human serum albumin colloid with $^{99m}$Tc (Nanocoll®) used in the present study was developed for bone marrow scintigraphy. According to the manufacturer, at least 95% of the particles have a diameter below 80 nm. In a previous study made at our institution, using gel filtration of one single batch of this radiocolloid, it showed a narrow peak at 31 nm (Kalin et al). This radiocolloid may be considered rather small, but was used in the present study as this was part of a national protocol for sentinel node scintigraphy.

## 4.5 PERTECHNETATE ($^{99M}TCO_4^-$)

Pertechnetate ($^{99m}TcO_4^-$) is the anion that is eluted from the $^{99}$Mo/$^{99m}$Tc-generator together with $Na^{2+}$. It is used to label different compounds in order to produce the desired radiopharmaceutical. The labelling step usually involves reduction of the heptavalent technetium by $Sn^{2+}$ to form a complex with the various ligands. The pertechnetate ion is small and largely handled by the human body as iodide. It is clinically used for thyroid scintigraphy and to depict ectopic gastric mucosa in Meckel's diverticulum. Consequently, the thyroid and stomach are visualized after administration of pertechnetate. Upon intravenous injection, the agent is rapidly distributed in the extravascular space and subjected to renal excretion.

# 5 MULTI-DRUG RESISTANCE

Cross-resistance to structurally unrelated anti-neoplastic drugs limits the success of multi-drug based chemotherapy regimens in malignancies. One type of multi-drug resistance (MDR) is caused by the transport of drugs over the plasma membrane mediated by adenosine triphosphate (ATP)-dependent transmembrane transporter proteins, $^{99m}$Tc (Ling). Conventional cytotoxic agents used in the treatment of breast cancer may be substrates of Pgp and MRP and thereby extruded from the cell. Since $^{99m}$Tc-MIBI is handled in the same way, the agent has been used to measure Pgp and MRP transport function. After an initial rapid tumour uptake of $^{99m}$Tc-MIBI, a correlation between the tracer efflux rate and the Pgp level in the tumour has been shown, and washout indices may be calculated to assess this activity (Del Vecchio et al 1997). The issue is touched in Paper 5, but is beyond the general scope of the study. All examinations with $^{99m}$Tc-MIBI were made early after administration of the tracer.

# 6 AIMS OF THE STUDY

The study intended to evaluate and to improve various radionuclear imaging methods and radiopharmaceuticals to be applied in breast cancer. The following aims were set up:

- To determine the clinical value of mammoscintigraphy with $^{99m}$Tc-MIBI as a complementary method to triple diagnosis, especially in patients with palpable breast lesions compared to patients with non-palpable breast lesions.
- To achieve an intraindividual comparison of the uptake of $^{99m}$Tc-MDP and $^{99m}$Tc-MIBI in large breast cancers.
- To evaluate examination with $^{99m}$Tc-MIBI in the assessment of neoadjuvant (preoperative) chemotherapy in large breast cancers at an early stage of treatment.
- To investigate if administration of a small pertechnetate activity ($^{99m}TcO_4^-$) in order to improve the anatomical localization at sentinel node scintigraphy in breast cancer reduces the detectability of radioactive lymph nodes.
- To investigate if mammoscintigraphy with $^{99m}$Tc-HMPAO can be used to image breast cancer and to compare it with $^{99m}$Tc-MIBI.

# 7 PAPER I

From above follows that $^{99m}$Tc-MIBI can be used to depict breast cancers. Its clinical application for this purpose varies much depending on established diagnostic routines in different countries and medical settings. Also the clinical status of the patients affects the use and value of a diagnostic modality. So are the studies of Khalkhali, the father of the lateral prone view at mammoscintigraphy, mainly made in Mexican immigrants in California, USA. These women in general have large breast and also large cancers at diagnosis. This is different to the average woman and standard procedures in Sweden.

Despite a high sensitivity of triple diagnostic procedure (TD), equivocal and inconclusive diagnoses occur why it sometimes is necessary to apply complementary methods. In a previous work at our hospital, the addition of mammoscintigraphy to TD led to a higher rate of cancer detection (Danielsson R, Acta Radiol 2000). Many studies have shown a high sensitivity and specificity of mammoscintigraphy for palpable breast cancers (Burak Z et al, Kao C-H et al, Lam WWM et al, Khalkhali I, 1995), while for non-palpable lesions the accuracy is less favourable (Aguilar J, Scopinaro F, Khalkhali I et al, 2000). The intention was to assess if adding mammoscintigraphy increases the cancer detection with special regard to lesions' palpability.

96 females with 119 breast lesions were examined with standard diagnostic procedures before surgery. Mammoscintigraphy was made in the lateral prone position together with an anterior acquisition and evaluated binary; a hot spot or not. At mammography, a standard 5 grade scale was applied. Sensitivity and specificity rates were calculated by defining diagnosis with score 1-3 as benign and 4-5 as malignant. When different modalities led to discordant results the final result was defined as the diagnostic group with the highest value. All lesions were excised and examined at histopathology. Sixty-five lesions were palpable and 54 were non-palpable. There were 83 malignant lesions, and 36 benign lesions. The palpable cancers (n=46) varied in size from 7 to 110 mm (largest diameter at histopathology), with a median of 22 mm. The non-palpable cancers (n=37) varied from 3 to 70 mm with a median of 14 mm.

In palpable lesions the sensitivity of mammoscintigraphy was 91.3% and higher than that of mammography (78.2%). TD showed higher sensitivity than mammography or mammoscintigraphy alone, and the sensitivity was further increased by the combination of TD and mammoscintigraphy (from 95.6% to 100%). When all three modalities of TD are concordant for malignancy, carcinoma is found in 100%. This means that adding of mammoscintigraphy in those patients will not be beneficial. However, in patients with results of TD concordant for benignancy (n=11), carcinomas were found in 2 patients (18.1%). Mammoscintigraphy was able to detect 2 more carcinomas, which were misinterpreted by the methods included in TD.

In non-palpable lesions the sensitivity of mammoscintigraphy was 78.3% compared to 89.1% for mammography. The sensitivity of the combination of mammography and fine needle aspiration cytology (double diagnostic procedure, DD) was the same as mammography alone, but increased by the combination of DD and mammoscinti-

graphy (from 89.1% to 97.2%). Concordant diagnoses of malignancy at DD were found in 100% and no additional carcinomas were detected by mammoscintigraphy. Among concordant diagnoses of benignancy at DD, 4 lesions were false negative. Mammoscintigraphy was able to detect 3 of these 4 carcinomas, resulting in a sensitivity of combining of DD with mammoscintigraphy at 97.2%.

According to our own and others' previous experiences, mammoscintigraphy is of limited value in lesions smaller than 10 mm (Bombardieri E, Danielsson R 1999,Taillefer R, 1995) and there is a strong correlation between the palpability of the lesions and the diagnostic accuracy of mammoscintigraphy (Mekhmandarov S). Even if the sensitivity of mammoscintigraphy in this study was lower for non-palpable than for palpable lesions (78.3% compared with 91.3%), this fact should be seen in the light of the histological size of the tumour. Thus, although a non-palpable lesion is not necessarily a small tumour, the majority of non-palpable tumours in the study was smaller than the palpable ones, for which reason alone a lower sensitivity of mammo-scintigraphy is to be expected. However, the addition of mammoscintigraphy increased the sensitivity for detection of breasts carcinomas in both palpable and non-palpable lesions. Mammoscintigraphy is therefore recommended as a complementary method to clinical-radiological-cytological evaluation in problem cases in both palpable and non-palpable breast lesions. This increase in sensitivity, however, is reached by costs of lower specificity, why avoidance of unnecessary biopsies cannot be expected, but all cancers will be detected.

# 8 PAPER II

The study was inspired by Piccolo et al reporting in 1995 that examination with $^{99m}$Tc-MDP could be used to image breast cancers. This prospective study based on a large number of patients gave rise to a great deal of attention. A cheap tracer, routinely applied to depict bone metastases, could be used to depict a primary tumour at the same occasion. Since a control group was included in their study, some kind of specificity could also be assessed, while no comparison with other radiopharmaceuticals for this purpose was made. Breast cancer uptake of $^{99m}$Tc-MDP has thereafter been confirmed by several other studies (Togni et al, Marwah et al, Massardo et al 2002a, Fawzy et al).

The obvious next step was to compare the tumour uptake of $^{99m}$Tc-MDP with that of $^{99m}$Tc-MIBI, the most commonly used agent for mammoscintigraphy. The study was performed as a paired (intra-individual) comparison in twenty patients. For ethical/dosimetrical reasons we were restricted to examine patients with locally advanced breast cancer. This, obviously, represents a patient selection. Each patient was examined with both agents within a short time interval using SPECT in the supine position, and wearing a brassière. All transverse sections showing a tumour uptake were added into one image and the net tumour uptake was compared to that of surrounding breast tissue. Also the maximum tumour uptake versus the surrounding breast tissue activity was calculated. A tumour uptake was observed at all examinations. In contrast to $^{99m}$Tc-MIBI, eight $^{99m}$Tc-MDP examinations showed increased uptake in normal breast parenchyma in addition to the tumour uptake. There was no significant difference in net tumour uptake between the two tracers and non-parenchymal background activity, but the maximum of MIBI was significantly higher than that of $^{99m}$Tc-MDP. In eight of the $^{99m}$Tc-MDP examinations with parenchymal activity, corresponding mammograms were necessary to identify the tumour uptake correctly. It was concluded that $^{99m}$Tc-MDP may provide similar images to $^{99m}$Tc-MIBI in post-menopausal women not receiving hormonal replacement therapy, while $^{99m}$Tc-MIBI gives better tumour depiction in other women. Since all patients had a locally advanced breast cancer, no conclusions regarding the specificity of $^{99m}$Tc-MDP in the diagnosis of breast cancer can be made.

As mentioned above, the uptake mechanism of $^{99m}$Tc-MDP in breast tumours is not known. In the report by Arslan et al, comparing breast cancer uptake of $^{99m}$Tc-MDP and $^{99m}$Tc-MIBI, there is a discussion on this issue to which little has to be added. In their study comprising 20 patients, there was no calcification at light microscopy, why a mechanism other than chemisorption of the radiopharmaceutical is probable. A wide spectrum of reliable mechanisms has been formulated including increased blood flow, inflammatory changes, modification of local metabolism ($Ca^{2+}$-content, pH) and collagen deposits. Other factors that may affect the uptake of bone-seeking radiotracers in breast cancers are thought to be changes in cell metabolism, enlargement of the interstitial space, cell wall damage and hormonal effects (oestradiol etc.). Another proposed mechanism is the pharmacological receptor theory concerning enzymes such as acid and alkaline phosphatases. The fact that the tumour uptake of $^{99m}$Tc-MDP reaches a peak already at 10-20 min after administration strengthens the possibility of a

different uptake mechanism than that of normal bone. In addition, from other studies it seems that $^{99m}$Tc-MIBI accumulation mainly reflects intracellular organelle while the accumulation of bone seeking tracers mainly reflects the extracellular component (Nishiyama et al).

There has been made a few similar comparisons. In the only comparative study published *before* the current study, based on only eight patients and reporting very few clinical details, the uptake of $^{99m}$Tc-MIBI in breast tumours was greater than that of $^{99m}$Tc-MDP (Barlow et al). In a study by Atalay et al, also reporting few clinical details, there was no difference of the sensitivity between the two tracers in the detection of breast cancer, while the specificity of $^{99m}$Tc-MIBI was higher. In the more substantial report including 20 patients by Arslan et al, early (5–30 min) and late (2$^{nd}$ hour) planar acquisitions were made using both tracers. The tumours had a mean size of 2.4 cm ∅. The evaluation was mainly qualitative. The authors found that early images with $^{99m}$Tc-MDP demonstrated equal sensitivity and higher specificity in palpable breast lesions than with early $^{99m}$Tc-MIBI imaging. On the other hand, a higher sensitivity was obtained with $^{99m}$Tc-MIBI than with $^{99m}$Tc-MDP in non-palpable malignant breast tumours. The largest series of comparison between the two tracers has been made in an international multicentre study comprising 47 patients (Massardo et al 2002b). The examinations were made as planar acquisitions, qualitative assessment only and the tumour size not being reported (*'a palpable breast lesion'*). In this study, $^{99m}$Tc-MIBI exhibited a higher sensitivity than $^{99m}$Tc-MDP in the characterization of malignant breast lesions, while the specificity was similar.

Comparisons between $^{99m}$Tc-MIBI and $^{99m}$Tc-HMDP ($^{99m}$Tc-hydroxymethylene diphosphonate, oxidronate) have also been performed. $^{99m}$Tc-HMDP is a radiopharmaceutical with chemical and diagnostical properties very similar to those of $^{99m}$Tc-MDP. In one study of 100 patients, although describing few clinical details, $^{99m}$Tc-MIBI showed a significantly better accuracy in the diagnosis of breast cancer for 'blind' reporting, while there was little difference between the agents in a 'consensus' clinical reporting context (McCauley et al). In another study of 50 patients, a higher sensitivity of $^{99m}$Tc-MIBI in the diagnosis of breast cancers with a size of 0.7–10.5 cm at surgery compared to $^{99m}$Tc-HMDP was reported (Nishiyama et al). In one study in 29 females comparing $^{99m}$Tc-HMDP, $^{99m}$Tc-Tetrofosmin and contrast enhanced MRI in the diagnosis of breast cancer, $^{99m}$Tc-HMDP is not recommended for this purpose (Lind et al).

Grossly summarizing these reports, which all are differently designed, there is no consistent superiority of bone seeking tracers compared to $^{99m}$Tc-MIBI in the diagnosis of breast cancer. This is confirmed also by the current study.

# 9 PAPER III

Since nuclear medicine represents functionally/biochemically based imaging, a potential also for tumour characterization and for therapy evaluation is obvious. In the current study the possibility to use $^{99m}$Tc-MIBI in order to depict an early therapy response at neoadjuvant (preoperative) chemotherapy of breast cancer was evaluated. Standard treatment of locally advanced breast cancer today consists of neoadjuvant chemotherapy (Bergh et al). Tumours that are >5 cm in dimension, invade the chest wall or skin, have fixed lymph node metastases or involve a large fraction of a small breast are considered to be locally advanced breast cancer. Neoadjuvant chemotherapy is made in order to improve outcomes in patients with high-risk breast cancer, why also smaller tumours with a high proliferative index and/or being oestrogen receptor negative may be subjected to such therapy in our department. The aim is to deliver a fast attack to presumed distant micrometastases, to prevent the emergence of drug-resistant cell lines and to shrink the primary tumour in order to improve operability, this sometimes also to allow primary breast reconstruction. Importantly, an advantage of neoadjuvant chemotherapy is also regarded to be the possibility of assessing the effectiveness of the chemotherapy on the intact primary tumour, assuming this response may be a marker of treatment outcome (Machiavelli et al).

The neoadjuvant chemotherapy regimen of locally advanced breast cancer in our department, even if 'individually tailored', consists of a combination of three drugs; 5-fluorouracil, epirubicin and cyclophosphamide (FEC-courses). Therapy evaluation at such treatment, as well as at chemotherapy in general, is usually made by assessment of tumour size. Irrespective if this is made by physical examination or by a radiological technique, size reduction of a tumour represents a slow and sometimes also an unspecific mechanism. There is a delay between onset of therapy and tumour shrinkage, and remaining fibrotic tissue cannot be discriminated from residual tumour tissue (Feldman et al, Moskovic et al). Consequently, more functional evaluation methods must be sought for, why trying nuclear medicine techniques is obvious. Examination with FDG-PET has for some reason been much more evaluated for this purpose than single photon tracers. It has been shown that reduced uptake of FDG in general precedes volume changes assessed by CT or MRI in tumours responding to therapy (Young et al).

Since $^{99m}$Tc-MIBI is the most commonly used agent for mammoscintigraphy, this radiopharmaceutical was evaluated as a functional tracer to depict the effect of neoadjuvant chemotherapy of breast cancer. The tracer has previously been surprisingly little used for this purpose. The investigation was made using SPECT. Planar acquisitions are adequate in order to study the detectability of a tumour at which the tumour-to-background activity ratio is fundamental. Comparisons of the uptake of single photon radiotracers between different studies represent a different situation and are more complex. Since absolute quantifications such as SUV-calculations, which are made at PET-examinations, cannot be made using single photon tracers, the uptake of a tissue or of an organ is usually compared with some reference tissue(s) unless only analogous (visual) evaluation is made. At breast examination, SPECT allows the

evaluation also of several different tissues/organs in contrast to the planar lateral prone view, which is commonly used at clinical mammoscintigraphy. Such acquisition shows only the breast background tissue in addition to the tumour activity. SPECT is also in principle more adequate for quantification of activity. The Chang correction for attenuation of photons is not ideal for the chest, since this algorithm assumes the body is homogeneous with water attenuation. It was used since it is our routine procedure and it allows the comparisons that were made.

The possibility at all to depict a therapy response by examination with $^{99m}$Tc-MIBI was first studied in 23 women with locally advanced breast cancer. Two identical SPECT-examinations were made; one before therapy with FEC-courses was initiated, and one after this was finished. The treatment was made according to clinical routine, which means that the therapy effect was followed by physical examination and mammography. The number of given courses varied between two and six. Semi-quantitative assessment of the therapy response was also made from hematoxylin-eosin stained sections of the finally resected tumour. Digital evaluation comparing the tumour uptake with the breast background activity and with that of the right lung was made. Analysis of the entire patient group showed that the uptake $^{99m}$Tc-MIBI is significantly reduced after finished neoadjuvant chemotherapy. The histopathological analysis of the finally resected tumour in the entire patient group also showed a significant therapy effect after finished chemotherapy. Surprisingly there was no correlation between the scintigraphic change between the two examinations and the therapy response as assessed by the pathologist.

A significant reduction of the $^{99m}$Tc-MIBI uptake after successful neoadjuvant chemotherapy of breast cancer is also reported by all similar studies using the same tracer, but performed at otherwise different technical settings. FEC-courses were used in one such study (Maini et al). In five studies more or less different chemotherapy regimens were used (Cwikla et al 1997, Mankoff et al, Tiling et al 2001, Schillaci et al, Sari et al). In four studies the chemotherapy regimen was not reported (Varrella et al, Villlanueva-Meyer et al, Cwikla et al 1999, Prats et al, Odharova et al).

It the next step it was studied if the same technique can be used to predict the effect of neoadjuvant chemotherapy at an early stage, already after one therapy cycle. This was the final aim of the study. Thirty women with locally advanced breast cancer were examined. Six of these were included also in the previous patient group. Comparing examination made in average 19 days after the first chemotherapy course with a base-line examination showed small, and no significant effects. Also in this patient group, histopathological analysis of the finally resected tumour in the entire patient group showed a significant therapy effect after finished chemotherapy.

Examination with $^{99m}$Tc-MIBI has very little been used to assess early effects of neoadjuvant chemotherapy in breast cancer. This is surprising, as the agent is commonly used for mammoscintigraphy. So far, there are three reports where this has been studied. In contrast to our findings, all these studies report a reduction of tracer uptake early at neoadjuvant chemotherapy. In the abstract by Villanueva-Meyer et al from 1999, outside that *'Some tumours showed a very early response, as early as after one cycle of chemotherapy . . . .'*, very few details of the study are reported. The

chemotherapy regimen is not described. Interestingly, the authors have not thereafter published their findings in any peer-reviewed journal (PubMed, February 2005). The report by Tiling et al from 2001 is more substantial. Repeated examinations were made with SPECT; one base-line examination, two during neoadjuvant chemotherapy and one examination after finished chemotherapy. Of seven investigated patients, six finally showed a complete or partial response. Five of these also showed a reduction of the tumour uptake of $^{99m}$Tc-MIBI when compared to the pulmonary uptake. At visual evaluation, there was a reduction of the tumour uptake in only two patients. The chemotherapy regimen was not the same as used by us. From the same group, another similar study but using planar acquisition and reporting nine patients appeared two years later (Tiling et al 2003). The clinical data regarding some of these patients are quite similar to those of their study from 2001. The neoadjuvant chemotherapy regimen was the same and the findings and conclusions are similar to those of their previous study.

The fact that a therapy response was not reflected by a significant reduction of the $^{99m}$Tc-MIBI uptake by the tumour after one therapy cycle represents the key finding of the current study. This turned out surprising and cannot be explained. In this restricted study, however, a number of variables that may influence the findings had to be kept constant. One such factor is the time between the first chemotherapy cycle and the second examination. The optimal time for this is not known. We tried to keep this as long as possible and therefore made the second examination the day before or at the same day as the second therapy cycle was given; in average 19 days after the first chemotherapy cycle. In a previous study very similar to this, but using PET and [$^{18}$F]-FDG and/or $^{11}$C-acetate, a response could be noted at examination made 6-13 days after the first therapy course (Jansson et al). The authors also conclude that the second examination should be made approximately 10 days after the first chemotherapy course. Varrella et al suggest that there may be a temporary reduction of tumour uptake of $^{99m}$Tc-MIBI following chemotherapy start, and which is independent of clinical response. In addition, there is evidence that breast carcinomas that fail to accumulate $^{99m}$Tc-MIBI have an altered apoptotic program due to the over expression of the anti-apoptotic protein Bcl-2 (Del Vecchio et al 2003). The expression of this is inversely correlated with the uptake in $^{99m}$Tc-MIBI in malignant lesions. A reduced apoptotic index and an increased Bcl-2 expression have also been found in residual breast cancer following preoperative chemotherapy (Ellis et al).

In the first patient group of the current study there was a lack of correlation between, on one hand, the activity reduction before and after finished chemotherapy and, on the other hand, the histopathological evaluation of the therapy response. This cannot be explained. Obviously, nuclear medicine and histopathology measure completely different properties. It should also be noted that the semi-quantitative scale used at the histopathological evaluation was originally developed to assess response at neoadjuvant chemotherapy of osteosarcoma (Picci et al). The scoring scale used in the report by Mankoff et al, and where a correlation between histopathology and reduction of $^{99m}$Tc-MIBI uptake after finished chemotherapy was reported, is not the same and less detailed than the scale currently used. Interestingly, is reported from a similar study by Cwikla et al in 1999, a clear lack of agreement in two patients between the reduction of $^{99m}$Tc-MIBI uptake and histopathological response after neoadjuvant chemotherapy of

breast cancer. As an explanation, the authors speculate in the development of P-glycoprotein. This may be correct although, as mentioned earlier, it is currently believed that Pgp enhances the extrusion of the tracer, rather than it influences the initial uptake.

# 10 PAPER IV

A 'sentinel' is a person or an animal set to guard a group of people (Websters New World Dictionary). The term 'sentinel node' was invented by Cabanas in 1977 to designate the first draining lymph node at penile carcinoma. By lymphangiography and anatomic dissections, the existence of a specific lymph node centre that appeared to be the primary site of metastases from the tumour was demonstrated.

The 'sentinel node concept' had its break-through with the report in 1997 on its application in breast cancer (Veronesi et al). This started a high enthusiasm as judged by many publications in the literature and the attention that was paid to this subject by editorials in major medical journals. The idea is that the lymphatic drainage of a tumour goes to one or a few sentinel nodes. These converge to 'secondary' lymph nodes and so forth; the tumour spread following this simple principle. Identification of the sentinel node/s and subjecting this to histological analysis provides a tumour staging since, according to this concept, a negative sentinel node implies there is no tumour spread 'downstream'. While most data have been collected from patients with breast cancer and malignant melanoma, there is an increasing interest also with regard to head-and-neck cancer, colorectal cancer, penile cancer and vulva cancer. The sentinel node concept allows more individually tailored surgery with obvious savings regarding human suffering and costs. At breast cancer, axillary lymph node detection can be avoided in the case of a negative sentinel node, this decreasing incision size and surgical morbidity as well as increasing postoperative function. Patients are becoming increasingly aware of the potential of this strategy and sometimes also request such surgery. Prospective studies of large patient groups and over a long time will eventually show the validity of the sentinel node concept.

So far, sentinel node scintigraphy has been used as a method to depict the functional anatomy, i.e. the regional draining lymph node(s). It is well known that lymph nodes completely filled up with metastatic tissue may not be visualized, this lowering the accuracy of the technique. Interestingly, it has recently been shown that lymph nodes partially involved by metastases from breast cancer may also show a greater colloid uptake than uninvolved nodes in patients where there are both involved and normal nodes (Canizales et al).

The sentinel node is visualized by the deposition of a blue dye at surgery or by the deposition of a radiopharmaceutical that is detected by a hand-held radiodetection probe at surgery. Combining these two methods gives the highest sensitivity. The dye-technique has remained rather constant while there are significant differences in practice relating to almost all aspects of the nuclear medicine technique. The major technical issues in sentinel node detection using nuclear medicine techniques are summarized in Table 2. Despite considerable differences of how the examination is clinically performed at various centres encouraging results are, strangely enough, usually reported regarding the possibility to detect the sentinel node/s.

**TABLE 2.**
**Technical issues in sentinel node detection using nuclear medicine techniques**

What radiopharmaceutical?
- Human serum albumin colloid
- Rhenium sulphide colloid
- Antimony sulphide colloid
- Sulphur colloid (filtered to reduce size)
- Stannous phytate (forming colloid upon injection)
- Human serum albumin (experimental)
- Polyclonal immunoglobulins (experimental)
- Modified dextran (experimental)
- Liposomes (experimental)

What size/size distribution of the colloid?

What injection site?
- Intradermal superficial to the tumour
- Subdermal superficial to the tumour
- Intratumoural
- Peritumoural
- Subareolar
- Periareolar

What injection volume?

Which activity (MBq's) administered?

Massage after injection or not?

How (or if) imaging?
- Dynamic
- Early imaging
- Early and late imaging

Skin marking or not?

One day or two day protocol?

How (or if) to depict the surrounding anatomy?
- Flood source behind the patient to define the body outline
- Waving a syringe with radioactivity behind the patient to define the body outline
- Moving line source behind the patient to define the body outline
- Flexible line source to define the body outline
- Radiation sources at specific anatomical structures
- Using internal scatter from the injection site to generate an image of the body

In practice, the role of the nuclear medicine physician today is to contribute to the detection of the sentinel node/s as efficiently and correctly as possible. The current study was concentrated on how to depict the surrounding anatomy. Isolated hot spots at gamma camera examination appear as a 'lighthouse in the darkness' and some kind of support is necessary to define the anatomy. From Table 2 follows that different techniques have been applied for this purpose.

In this retrospective study, the number of sentinel nodes detected at gamma camera imaging and at subsequent surgery were compared between two groups of females with breast cancer. Forty-seven patients had 25 MBq of pertechnetate i.v. and a subcutaneous injection of radiocolloid superficial to the tumour. 41 patients did not have pertechnetate. The patient groups were otherwise comparable. Gamma camera examination (anterior and lateral views) was made 2-3 hours after administration the radiopharmaceutical(s). There was no difference in the number of detected lymph nodes between the patient groups, neither at subsequent gamma camera examination nor at surgery.

For obvious reasons it could not be studied if, or to what extent the current technique really simplifies the localization of the radioactive lymph nodes or how it behaves compared to other methods for this purpose. Consequently, in addition of describing the technique, the report concentrated of the possibility that the administration of pertechnetate may reduce the detectability of lymph nodes with a low uptake, by a reducing the signal-to-background activity ratio. The aim was not to compare the number of detected sentinel nodes between scintigraphy and surgery. Most techniques previously used only depict the body contour, while the current technique also highlights the chest with the thyroid and stomach, which usually are included in the field of view. In practice, this should prevent confusion in image orientation and make the images easy to read at subsequent surgery.

The study allows the conclusion that administration of 25 MBq pertechnetate in order to ensure an adequate depiction of the anatomical background does not reduce the detectability of the sentinel nodes, neither at gamma camera imaging nor when using a hand-held probe at surgery.

# 11 PAPER V

The diagnosis of breast cancer in Sweden is based on triple diagnosis (TD). Sometimes complementary diagnostic techniques are required, and mammoscintigraphy has confirmed its value for this purpose. While many radiopharmaceuticals have been investigated as tumour seeking agents in breast cancer (see above), $^{99m}$Tc-MIBI and $^{99m}$Tc-Tetrofosmin are the only single photon emitting tracers used for this purpose in clinical practice. A broadening of the spectrum of agents that may be used for this purpose would strengthen the role of mammoscintigraphy. The purpose of this study was to explore if $^{99m}$Tc-HMPAO is suitable to depict breast cancer and to compare this with $^{99m}$Tc-MIBI. $^{99m}$Tc-HMPAO is a common agent for the study of regional cerebral blood flow (rCBF), but there are a number of reports describing occasional uptake in various tumours. Uptake by human breast tumour cell lines is also reported. The study was made in clinical patients with breast tumours ≥1 cm.

A kinetic study was first made in 20 patients. This was required as the uptake dynamics in tumours by $^{99m}$Tc-HMPAO were not fully elucidated. The examination was made as lateral prone planar acquisitions in order to allow the dynamic study. The tumour uptake increased during 10 min and was thereafter practically stable. The registration lasted for 30 min, but a late significant redistribution of the tracer seems very unlikely. In the second part of the study, an intra-individual comparison of the tracer uptake was made in 21 patients. For practical reasons this was also made in the lateral prone position. SPECT is more accurate for quantification, but planar acquisitions are sufficient since the current question concerned the detectability of a breast tumour. This has to be made by assessing the tumour uptake versus the surrounding background activity, and the lateral prone planar view is the common position at clinical mammoscintigraphy.

All tumours (altogether 43 tumours in 39 patients) were visualised by $^{99m}$Tc-HMPAO. There was one false positive uptake that cannot be explained. $^{99m}$Tc-MIBI failed in depicting four tumours, also this for unknown reasons. On the *group* level, there was no difference between the tracers with regard to the tumour uptake, while the *intraindividual* agreement between the tracers was restricted (intra-class correlation coefficient, ICC=0.49). The specificity in the detection of breast cancer by $^{99m}$Tc-HMPAO cannot be elucidated since all patients had known tumours.

The uptake mechanism of $^{99m}$Tc-MIBI has been studied in various organs outside the myocardium as well. In contrast to this, the uptake mechanism of $^{99m}$Tc-HMPAO outside normal brain tissue is poorly known. In most reports describing uptake of this agent in tumours outside the brain, the uptake mechanism has been assumed to be the same and reflecting 'perfusion'. This may be discussed as the early uptake dynamics in the current study was not the same as reported for the brain. The brain uptake of $^{99m}$Tc-HMPAO is obviously more rapid than found in breast tumours in the current study.

The restricted *intraindividual* agreement between the tracers confirms different uptake mechanisms of the two tracers. This finding may open up possibilities to assess

different tumour characteristics *in vivo*. Several different tumour properties may be discussed, although this issue is far beyond the scope of the present study. Nevertheless, the fact that the uptake of both agents is based on mechanisms believed to be involved in resistance to anti-neoplastic drugs is challenging. Intracellular fixation of $^{99m}$Tc-HMPAO is considered to be based on interaction with glutathione. There is a correlation between elevated cellular concentrations of glutathione and resistance to alkylating agents and cisplatin. These drugs may be inactivated through conjugation with intracellular glutathione by catalysation of glutathione S-transferases, and thereafter extruded from the cells. Another type of drug resistance is caused by an efflux of drugs from tumour cells mediated by ATP-dependent transmembrane transporter proteins, P-glycoprotein (Pgp) and multidrug-resistance protein (MRP). The washout rate of $^{99m}$Tc-MIBI from tumour cells is positively correlated to the expression of these proteins.

# 12 GENERAL REMARKS

## 12.1 DEVELOPMENT OF RADIOLOGY AND NUCLEAR MEDICINE

The technical development in radiology has been considerable during the last three decades. Until the mid-seventies, diagnostic radiology was in practice restricted to planar imaging including fluoroscopy, sometimes enforced by linear tomography and angiography. Today, a radiological department looks completely different, and a majority of the diagnostic information is achieved by ultrasonography (US), computed tomography (CT) and magnetic resonance imaging (MRI). These computerised techniques provide very detailed sectional information based mainly on different physical properties of the tissues examined. Contrast agents specifically developed for the different modalities have also contributed to increase their versatility. By providing earlier, more exact and also more detailed diagnostic information, this development has considerably influenced the entire field of clinical medicine. The impact has been most pronounced in the various surgical entities and in oncology. A thorough anatomical mapping of the disease extent by adequate radiological technique(s) is today usually required to allow optimal surgery of most parts of the body. Diagnostic surgery is today seldom performed and various interventional radiological procedures have replaced certain surgery.

Despite this apparent development of radiology, also nuclear medicine has undergone a continuous development, and the diagnostic value of this modality has kept its position, or even increased in clinical medicine. This is to some extent because of technical improvements, but the development of better and new radiopharmaceuticals has also been important. SPECT and PET have certainly emerged as powerful diagnostic methods while, on the other hand, the gamma camera has in principle remained unchanged. The collimator, which constitutes the image-forming unit at single photon imaging, limits the image quality by providing a restricted spatial resolution and detection efficiency. This development, thus, emphasizes the functionally basis of imaging in nuclear medicine and the importance of the radiopharmaceutical. Without this, there will be no images.

## 12.2 DIFFERENCES BETWEEN RADIOLOGY AND NUCLEAR MEDICINE

Radiological examinations have emerged as being excellent for anatomical imaging with a very high spatial and contrast resolution, while the possibility to assess the nature behind a lesion, as well as the functional information is restricted since all radiological techniques are based on physical properties. The image signal obtained at radiography and CT reflects differences of electron density, ultrasonography is based on differences of acoustic impedance between different media, and the signal at MRI is mainly based on differences of proton density. Consequently, the interpretation of such examinations includes an 'intellectual jump' from physics to biology which sometimes restricts biological and functional conclusions. Nevertheless, based on a combination of experience and sense, very accurate diagnoses may be obtained. Experience and sense is also required for proper interpretation of nuclear medicine examinations, but the

signal from a radiopharmaceutical is always based on some kind of biological property. As earlier mentioned, this makes radiology and nuclear medicine complementary examination techniques. An obvious illustration of this is the rapid word-wide increase of the number of combined PET-CT devices for clinical purposes.

In order to exploit the complementary value of nuclear medicine and radiology, both techniques have to adopt to the 'ever changing' reality. Duplicating examinations by different modalities are in most cases not required, why certain scintigraphic examinations have been discontinued and other also will be discontinued in the future. The apparent development is that nuclear medicine examinations are focused on biological and biochemical imaging, and anatomical imaging as well as negative imaging is gradually given up. One example of this is that liver/spleen scintigraphy using radiocolloids has been discontinued in favour of various modern radiological modalities. Also the use of lung scintigraphy in the diagnosis of acute pulmonary embolism is today rapidly being replaced by helical CT. This process, however, is counterbalanced by the rapid development of new radiopharmaceuticals. Nuclear medicine has taken advantage of molecular biology. Molecular imaging, allowing specific depiction of very small amounts/concentrations of chemical compounds or receptors, is today a reality. Unfortunately, all promising radiotracers cannot be converted into commercial radiopharmaceuticals because of high costs, restricted markets and excessive demands by the regulatory authorities in many countries.

PET is not only a technology offering an improved imaging but has also given rise to a series of radiopharmaceuticals fundamentally different to single photon emitting tracers. The principal advantage of PET-tracers is that e.g. carbon, nitrogen and oxygen can be labelled, this allowing the use of 'biological' compounds as tracers. This possibility represents a considerable potential both for scientific purposes and for clinical examinations. Despite this, the most clinically used PET-tracer is today FDG which does not represent a 'biological' compound.

## 12.3 NUCLEAR ONCOLOGY

The functional information provided by nuclear medicine examinations makes the technique being increasingly used for tumour diagnosis and tumour evaluation. 'Nuclear oncology' is today an established entity with a growing attention worldwide. Accordingly, the number of contributions in this field increases every year at the two large international annual events in nuclear medicine; European Association of Nuclear Medicine Congress and Society of Nuclear Medicine Annual Meeting.

Principal applications of nuclear oncology are:

- Detection and staging of tumours (including recurrences)
- Characterisation of tumours/lumps
- Therapy evaluation

## 12.4 DETECTION AND STAGING OF TUMOURS

In clinical reality most tumours are not detected by nuclear medicine examinations, but rather by physical examination, radiological examinations, endoscopy or surgery. The use of scintigraphy for this purpose will undoubtedly increase with an increasing awareness and experience of the growing nuclear medicine techniques. There is also an obvious tendency in our department to use FDG-PET for staging in patients with known or recurrent tumours. The 'ideal' tumour depicting radiopharmaceutical shows the tumour without any irrelevant or interfering activity. The advantage of this principle is obvious in contrast to radiological modalities, where only a very small fraction of the image information represents the tumour, and considerable effort and time have to be spent in order not to overlook pathological changes.

So far, however, the 'ideal' tumour depicting tracer does not exist. All tracers used for this purpose are based on some kind of normal mechanisms or biological properties, why they are not homogenously distributed throughout the body. This is true for 'generalist' tracers used to depict e.g. cell metabolism, amino acid transport and synthesis of proteins, nucleic acids and membrane components, amenable to use in a wide variety of tumours, as well as for 'specific tracers' used to depict e.g. receptor expression, cell hypoxia and bone metabolism. Consequently, specificity often has to be added by the reading nuclear medicine physician supported by experience and a thorough clinical knowledge about the patient examined including availability of current radiological examinations.

In paper I, the value of mammoscintigraphy by $^{99m}$Tc-MIBI in the diagnosis of breast cancer was studied. As not uncommon when adding a scintigraphic examination, mammoscintigraphy in addition to TD increases the sensitivity but reduces the specificity. From this follows that mammoscintigraphy by $^{99m}$Tc-MIBI should be used as an adjunct to other diagnostic techniques taking advantage of its sensitivity in cases where exclusion of malignancy is important. There is a field of application for this examination e.g. in patients with dense breasts, breast implants and after breast surgery. In Sweden, the technique is underused for this purpose.

Paper II also deals with tumour detection. The report shows that there is no advantage for the detection of large breast cancers by $^{99m}$Tc-MDP compared to $^{99m}$Tc MIBI. The value of studies with a negative outcome may be discussed. The study is robust, and *comparative* studies of this type were important to make after the report by Piccolo et al in 1995 to which a great deal of attention was paid. In addition, even if bone scintigraphy can be used to depict a primary tumour at the same occasion, this fact is seldom possible to take advantage of in the clinical situation. When a patient with breast cancer comes to bone scintigraphy, the tumour diagnosis is usually already established.

In paper V, $^{99m}$Tc-HMPAO, a well-known radiotracer, but rarely used for tumour depiction was applied for breast cancer depiction with a sensitivity at least as good as $^{99m}$Tc-MIBI. The uptake mechanism is as unspecific as for $^{99m}$Tc-MIBI, and only future larger studies will show the specificity of the tracer for this purpose. The finding

illustrates, however, that radiopharmaceuticals sometimes may be used for completely different purposes than they originally were intended for. Another example of this is the use of $^{99m}$Tc-MIBI for breast cancer diagnosis.

## 12.5 CHARACTERISATION OF TUMOURS/LUMPS

Even if the uptake of a radiopharmaceutical reflects a normal mechanism, uptake (or not) of a known lesion in a given clinical situation may be very informative. This use of nuclear medicine examinations is important and represents a future trend in nuclear oncology. Many examples may be mentioned. Uptake of FDG in a residual tumour mass at CT or MRI after chemotherapy for lymphoma indicates remaining active tumour, requiring further treatment. Octreotide-therapy of a tumour expressing somatostatin-receptors may be successful. This may be assessed by examination with $^{111}$In-pentetreotide (OctreoScan®). In patients with phaeochromocytoma or in children with neuroblastoma, radionuclide therapy with [$^{131}$I]-mIBG may be successful if there is uptake at diagnostic examination with [$^{123}$I]-mIBG.

This application of nuclear medicine for characterisation of tumours/lumps is restricted in the present study. Nevertheless, the findings reported in paper III suggest that combined mammoscintigraphy with $^{99m}$Tc-MIBI and $^{99m}$Tc-HMPAO may be used for tumour characterization. This, since the uptake mechanism is different and the agreement between the two tracers is restricted. There is so far no possibility to tell how or for what combined examination with these radiopharmaceuticals can be applied for any clinical purpose.

## 12.6 THERAPY EVALUATION

Using nuclear medicine techniques in order to early assess response at chemotherapy of tumours is an attractive approach. Morphological changes constitute slow signs of response, while functional changes are rapidly induced in case of a therapy effect on the tumour. During the last years the number of reports suggesting the use of PET for this purpose has rapidly grown, although there is yet no consensus on how to apply the technique in clinical praxis. In contrast to this, there are very few reports using single photon tracers for this purpose.

The study in paper III was made since $^{99m}$Tc-MIBI is available at all nuclear medicine laboratories to an acceptable cost. It is also the most common agent for clinical mammoscintigraphy. The fact that a therapy effect could not be detected after one therapy cycle was surprising and cannot be explained. It is also in contrast to the small number of previous reports in this field. The current study is, however, more substantial than the previous reports and the findings have to be trusted. It may be that examination with $^{99m}$Tc-MIBI after two therapy cycles would show an effect. This would certainly also represent a diagnostical improvement. As mentioned, examination after each cycle was not possible for dosimetric reasons, why this question is beyond the scope of the current report.

## 12.7 PAPER IV

Sentinel node scintigraphy does not represent direct tumour imaging, but is part of nuclear oncology. In this report a nuclear medicine method is used to depict functional anatomy. Mainly because of the deposition of the tracer in a specific region, but also because of the lack of 'irrelevant information' as desired at many nuclear medicine examinations, the anatomical orientation is difficult. In principle there are many ways to achieve such complementary information. One example of this is the increasing use of combined PET-CT devices. In this case a very simple technique was used without interfering with the detection efficacy of regional lymph nodes.

# 13 CONCLUSIONS

- Scintimammography with $^{99m}$Tc-MIBI has an additional value to the standard triple diagnosis. It increases the sensitivity for the detection of both palpable and non-palpable breast cancers, but decreases the specificity.

- $^{99m}$Tc-MDP can be used in the detection of large breast cancers. Outside that it may be performed as part of a clinical bone scintigraphy, there is no advantage compared to examination with $^{99m}$Tc-MIBI.

- $^{99m}$Tc-MIBI can be used to evaluate neoadjuvant chemotherapy effect after finished treatment, but it is not reliable as a response detector after one therapy cycle.

- Sentinel node scintigraphy in breast cancer can be performed after i.v. injection of 25 MBq pertechnetate together with the routine procedure. It provides a better anatomical orientation without affecting the sentinel node detectability.

- $^{99m}$Tc-HMPAO is a feasible breast cancer marker. It may be at least as sensitive as $^{99m}$Tc-MIBI, while the specificity cannot be assessed. It may be that the combination of examination with $^{99m}$Tc-HMPAO and $^{99m}$Tc-MIBI can be used for biological tumour characterization.

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