PhD theses

The Role of Nuclear Medicine in the Preoparative Diagnostics of Malignant Breast Tumours

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University of Pécs, 2012

Introduction

The incidence of breast tumours has been rising from year to year. This is the most common cause of cancer related death in women. According to breast cancer mortality, Hungary is among the less fortunate countries in Europe. Applying mammographical screening a significant improvement in survival of breast cancer patients can be demonstrated. Recently stagnation moreover decrease has been observed in the yearly mortality rate, not only in Europe but in Hungary too, which is primarily due to early diagnosis and organized screening. The significant decrease in breast cancer mortality can be expected only after a long-term screening program and high participation rates. Correct preoperative diagnostics plays an essential role in the operative and postoperative therapy of breast tomours. There is no ideal diagnostic method; each method has its own advantages and disadvantages. Besides the physical examination mammography and ultrasound are the most important methods, however setting up the diagnosis rarely includes MRI or nuclear medicine methods. The main difficulties in diagnostics are mastopathic, dense breasts (adenosis, hyperplasia) which are typical of premenopausal women. On the other hand the density of the breast increases in about one third of women receiving hormone replacement therapy, as well on mammographic images, which makes the diagnosis uncertain. Due to this fact, further diagnostic procedures will be necessary. Nuclear medicine methods may be helpful in such cases, however the clarification of the real role and significance of these methods necessitates additional examinations. Accumulation of radiopharmaceuticals in various tumours has been widely studied. Besides labelled monoclonal antibodies and receptor ligands (111In-somatostatin, 18F-estradiol) radiopharmaceuticals with non-specific tumour uptake are more fraquently used: 18F-FDG (fluorodeoxyglucose), 201TlCl, and because of its ready availability 99mTc-MIBI (methoxyisobutylisonitrile), 99mTc-tetrofosmin as well as 99mTc-MDP (methylene diphosphonate). While 99mTc-MDP is used for bone scintigraphy, 99mTc-MIBI and 99mTc-tetrofosmin are principally applied in myocardial perfusion imaging. The latters are small cationic, lipophilic complexes which accumulate intracellularly in mitochondria, consequently in cells which are rich in mitochondria and have an increased metabolism. It is well known that 99mTc-MIBI accumulates in malignant breast tumours with various intensity. The intensity of accumulation depends on a number of factors. Recent research has demonstrated that

the intensity of uptake is also affected by blood supply for tumours as well as by important prognostical factors such as multidrug resistance.

In the prognosis and therapy of breast cancer –in addition to the early and correct tumor detection- tumoral involment of axillary lymph nodes is an important aspect. Decades earlier attention was already drawn to the importance of the lymphatic drainage of the breast and to the prognostical role of lymph node status. With the spread of education and mammographic screening there has been a significant increase in the proportion of lymph node negative cases diagnosed in an early stage. Conventional surgical methods meant axillary block dissection in all cases. However the removal of metastasis-free lymph nodes is unnecessary. The axillary block dissection may be associated with significant morbidity (seroma formation, lymphoedema, infection, pain in the upper arm, paraesthesia, limitation of movement) whereas at the same time the result of routine histological examinations of the removed lymph nodes is in 7-33% of cases false negative. The sentinel lymph node (SN) biopsy has been developed to exclude these disadvantages. SN is a special lymph node of the drained region. A lymph vessel, originating from the primary tumour, leads to this lymph node; thus this is the most common site of the appearance of lymphogen metastases. With its removal and histological examination it is possible to decide if regional block dissection is necessary or not. SN scintigraphy and biopsy are minimally invasive interventions, which make it possible to avoid axillary block dissection in lymph node negative cases and it allows for saving the option of histological staging of the lymph node status as well. As a result of targeted, more detailed, pathological examination of the SN, lymph nodes diagnosed negative using routine techniques may result in a positive diagnosis. However problems can emerge during this method: e.g. SN is not labelled or SN is false negative in skip metastases. If the histological examination confirms lymph node metastases another operation will be necessary as a second step.

Objectives

1 Establishment of diagnostic value, applicability and indication of 99mTc-MIBI and 99mTc-MDP scintimammographies in the preoperative diagnosis of breast cancer patients.

2 Comparison of the performance of 99mTc-MIBI and 99mTc-MDP scintimammographies.
3 Clarification of the connection between the intensity of 99mTc-MIBI uptake and tumour invasiveness.
4 Determining the role of 99mTc-MIBI SPECT/CT in the follow-up of neoadjuvant therapy of breast tumours.
5 Establishing the performance and safety of SN scintigraphy.
6 Choosing the appropriate injecting technique of radiopharmaceutical.
7 Comparison of SN detected through the peritumoral, US guided administration of radiopharmaceutical and of SN detected through periareolarly administered patent blue dye, and the comparison of these methods.
8 Examination of suitability of peritumorally administered radiopharmaceutical as ROLL (Radioguided Occult Lesion Localisation).
9 Establishing the diagnostic performance of targeted preoperative SN FNB (Fine Needle Biopsy).
10 Elimination of false negative results caused by skip metastases.

Scintimammographies

I 99mTc-MIBI and 99mTc-MDP scintimammographies in the preoparative diagnostics of breast tumours

Patients and methods:

Between 1994 and 1996 the preoperative examination of 105 patients (103 females and 2 males) was carried out, in which, based on the physical examination and/or mammography, malignant breast tumors were suspected. The average age of patients was 57 years, the range was 28 to 75 years. Two patients were diagnosed with bilateral breast tumors. All patients underwent 99mTc-MIBI scintigraphy before the surgery while 99mTc-MDP scintigraphy was performed in 98 cases. Radiopharmaceutical was injected i.v. in both cases, into the cubital vein, on the contralateral side of the tumour, if it was possible, the administered radioactivity amounted to 740 MBq. Following administration, chest SPECT imaging was carried out (Multispect II, Siemens), 10

minutes later in the case of 99mTc-MIBI and 2 hours later in the case of 99mTc-MDP, activating two detectors, using ultra high resolution collimators, 180º rotation, 32 steps and 30 sec/step. In 22 patients in the case of MIBI the chest SPECT imaging was repeated later, 2 hours after the injection of radiopharmaceutical. On the one hand the accumulation of radiopharmaceutical in the tumour and axillary region, on the other hand in the case of 99mTc-MIBI the intensity of accumulation was examined.

Four groups were indentified based on the intensity of 99mTc-MIBI accumulation of the tumour:

Group 1: tumours with no visible overactivity,

Group 2: accumulation of very low intensity which could be differentiated from the background activity,

Group 3: more intensive accumulations, with less intensity than that of the heart,

Group 4: overactivities reaching the intensity of the heart, or exceeding it.

In each case the final diagnosis was made based on the histological examination, and TNM classification of patients was also performed, taking it into account. In 68 cases the histological examination was supplemented by the examination of vascularisation of the malignant tumour as well as by immunohistochemical staining (CD 34 antibody), suitable for the detection of endothelial cells. Several slices were prepared of the tumour. In each patient the number of cross-sections of small vessels was determined using several slices multiplied by 400, altogether in 20 visual fields, and then the average value for one visual field was calculated. Statistical analysis was carried out when processing our results: The sensitivity, specificity, positive and negative predictive values and validity of individual methods were determined. Spearmann rank correlation and Kruskal-Wallis test were used to investigate the correlation between the size of the tumour and vascularisation and the intensity of MIBI accumalation.

Results:

Histological examination confirmed malignant tumours in 92 cases of 107 breast tumours in the 105 patients examined. The majority of these tumours were ductal carcinomas (n=79), the number of lobular (n=7) and of tubular (n=3) carcinomas was much lower, while medullar and mucinosus carcinomas and sarcoma phylloides were confirmed in 1 case each. Tumours appeared to be benign in 15 cases. The histological findings confirmed mastopathy in 8 cases, fibroadenoma 6 cases and intraductal papilloma in 1 case. MIBI accumulated in 4 cases in mastopathy and 2 cases in

fibroadenoma, and the results are basically the same in relation to MDP. In 64 cases, that is in 73% of malignant tumours examined using both methods, both radiopharmaceuticals showed accumulation in the tumour. It is noteworthy, however, that when we examined the malignant tumours of premanopausal female patients (n=15), the proportion of MIBI+/MDP- was relatively high (5 cases which amounts to 33%). In addition to benign and malignant tumours, overactivity in the contralateral breast with no tumour was detected in 8 cases in MIBI and 19 cases in MDP. In these cases the overactivity was of low intensity and repeated mammography, breast US and biopsy indicated mastopathy. It should be noted however that in one patient with bilateral breast tumour, it was scintimammography, that detected contralateral tumour. Based on the histological results, the diagnostic effectiveness of 99mTc-MIBI scintimammography proved to be better than that of 99mTc-MDP scintimammography.

	99mTc-MIBI	**99mTc-MDP**
sensitivity	87%	81%
specificity	88%	77%
positive predictive value	85%	75%
negative predictive value	90%	83%
validity	88%	79%

Diagnostic effectiveness of 99mTc-MIBI and 99mTc-MDP scintimammographies in the diagnostics of breast tumours

In this study 14 patients of 105 had higher density on their mammographic scans, 8 of them had malignant and 6 of them had benign breast disease. The results of 99mTc-MIBI scintigraphy were positive in 7 malignant cases of 8 and in 2 of 6 patients with benign breast tumours. However, the results of 99mTc-MDP scintigraphy were positive in 4 cases of 8 malignant tumours, and in 2 cases of 5 benign tumours (in 1 benign case MDP scintimammography was not performed). The diagnostic effectiveness of 99mTc-MIBI also proved to be better than that of 99mTc-MDP in this patient group. Although because of the low case number of the latter patient group, the accurate evaluation of the

numerical data is limited; the difference in effectiveness between the two methods is obvious. It can be noted that with the increase in tomour size the incidence of false negative cases decreases when using both radiopharmaceuticals. The MIBI negativity of mucinosus carcinoma arises from its histological structure, high mucin content, gelatinous consistency and therefore relatively low mitochondrium content. In patients with axillary lymph node metastases 99mTc-MIBI scintigraphy showed accummulation in all cases in the breast cancer and there was only 1 false negative case with 99mTc-MDP, as well. In 22 patients, examined both in the early (10 minutes after injection) and delayed (2 hours after injection) phases, the changing of the intensity of 99mTc-MIBI uptake, comparing the early and delayed scans, showed the following results: in 9 patients no changing was detected, in 10 patients the intensity of uptake decreased on the delayed scans, while in 3 cases a minimal increase in accummulation was observed on the delayed scans. In these cases the delayed scans (2 hours after injection) did not change the results of diagnostical effectiveness of 99mTc-MIBI scintigraphy. In tumours, which were classified according to the intensity of 99mTc-MIBI uptake into 4 groups, there was a positive correlation between the largest tumour diameter established by histology and the intensity of uptake. Statistical analysis could be performed only in ductal carcinomas which had a relatively large case number. While in cases with no visible MIBI uptake, the mean tumour diameter amounted to 8.4 mm, in tumours with high activity, which reached the activity of the heart or exceeded it, this was 25.3 mm. Because of the small case number, in the other histological types averaging was meaningless, but the tendency in lobular carcinomas was the same. However we cannot leave out of consideration that there are large tumours of 60 mm, without 99mTc-MIBI accumulation and also small tumours (8 mm), with intensive uptake, that means that the scattering is high. Because the diameter data showed no normal distribution, Sperman's rank correlation and Kruskal-Wallis test were performed for statistical significance analysis. The value for Spearman's rho was 0.531 ($n=79$, $p < 0.001$). There was also a positive but low correlation between the intensity of accumulation and the number of cross-sections of vessels per visual field. In the detection of axillary lymph node matastases the results are less straightforward. Of 35 histologically proved lymph node metastases only 18 (51%) showed 99mTc-MIBI uptake and only 2 (6%) MDP uptake.

II 99mTc-MIBI SPECT/CT in the follow-up of the effectiveness of neoadjuvant therapy in breast cancer (preliminary experience)

Patients and methods:

8 breast cancer patients (mean age 58.5 years, the range was 40 to 79 years) were examined, with the indication of neoadjuvant chemotherapy. The core biopsy confirmed ductal carcinoma in 7 patients, and an undifferentiated tumour with high malignity in addition to mastitis in 1 patient. Before the initiation of the neoadjuvant therapy (Docetaxel-Epirubicine), and after the cycles III and VI 99mTc-MIBI scintigraphy was performed. After the injection of 740 MBq 99mTc-MIBI, early (10 minutes) and delayed (60 minutes) planar scans of the chest and axilla were performed, which were completed with whole body scan and SPECT/CT of the chest. We examined the intensity and the change in intensity of 99mTc- MIBI accumulation both in the breast tumour and also in axillary lymph nodes, as well as the appearance of further pathological accummulations. Quantitative analyses on SPECT/CT slices (VOI, maximum value) and calculation of activity ratio of the tumour and contralateral breast, as well as of pathological lymph nodes and contralateral axilla were carried out. The change in uptake intensity, tumour and lymph node size (CT) and postoperative histological findings were compared.

Results:

In 1 patient, who had mastitis and undifferentiated tumor with high malignity, there was a mild progression in tumor size and intensity of 99mTc-MIBI accummulation after the cycle III. The therapy had been stopped, then mastectomy was performed and the histology confirmed extraosseal osteosarcoma. In 1 case the baseline SPECT/CT scan found a pathological mediastinal lymph node and lung metastases, which was an indication for changing the therapy. In 1 case because of renal failure the planned neoadjuvant therapy could not be started. In 5 cases the intensity of 99mTc-MIBI accummulation showed a significant reduction or rather negativity already after the cycle III of therapy. In 5 patients pathological axillary lymph nodes were detected by mammography and breast US, which were confirmed by 99mTc-MIBI planar and whole body scans. However chest SPECT/CT showed pathological lymph node accumulation in 7 patients, in 2 cases with normal lymph node size. Five patients were operated on after the cycle VI. The SPECT/CT results before and after neoadjuvant therapy (cycle

VI) compared to the clinical and histological (after surgery) results are summarized in the following table. In the 5th patient histology confirmed significant tumor regression with tumour cell degeneration. The 4th patient, who had a relatively high lymph node activity ratio after cycle VI, was diagnosed with reactive hyperplasia.

patient	clinical/ hystological stage	SPECT/CT											
		Tumour						Lymph node					
		size (mm)		MIBI				size (mm)		MIBI			
				visually		act. ratio				visually		act. ratio	
		before	after	before	after	before	after	before	after	before	after	before	after
1	cT2N1 ypT3N2	30	3	poz.	neg.	5,82	1,38	28	<10	poz.	neg.	4,13	1,5
4	cT2N0 ypT2N0	45	5	poz.	neg.	4,33	1,92	<10	<10	poz.	neg.	1,82	1,77
5	cT2N1 ypT1cN1	50	0	poz.	neg.	7	0,86	40	10	poz.	neg.	3,18	0,85
6	cT2N0 ypT1cN0	35	20	poz.	poz.↓	5,2	2,7	10	<10	poz.	neg.	1,42	0,79
8	cT1cN1 ypT0N2a	30	0	poz.	neg.	2,77	1,15	16	<10	poz.	poz.↓	2,31	1,28

SPECT/CT results before and after neoadjuvant therapy (cycle VI) compared to the clinical and histological (after surgery) results

Sentinel lymph node scintigraphy

I. Introduction of the method, preliminary experience

Patients and methods:

Between November 1999 and February 2001 the examination of 54 female patients was carried out. The average patient age was 56.9 years, the range was 41 to 82 years. Two patients were diagnosed with bilateral breast tumours. Since our primary aim was gaining experience, we examined patients with large tumours, too, and also patients with axillary lymph node metastases, confirmed preoparatively by cytology. Sentinel lymph node scintigraphy was performed in all patients the day before the operation. The radiopharmaceutical (Senti-scint, Medi-Radiopharma, Budapest) was injected

peritumorally in 4 portions (4 x 15 MBq 99mTc-Senti-scint in 0,2 ml) using US guide. Anterior and lateral planar scans (with collection of 500000 counts) were performed 1 and 3 hours following the injections of the chest and axilla in question. In the first 28 cases also 24 hour scans were performed. The 24 hour scans brought extra information in 2 cases: when on the 1 and 3 hour scans no sentinel lymph node appeared and it became detectable only on the 24 hour scan. After that only 1 and 3 hour scans were performed routinely and 24 hour scans were needed only in 2 cases. The anterior and lateral projections of the SN were marked on the skin. Before the scintigraphy and after the marking, US examinations were carried out, observing selectively the lymph nodes. The operation was carried out the next day. For the identification of SN, an intraoperative detector was used (C-Track, Europrobe, Navigator). After the removal of SN, in all cases axillary block dissection and histological examination was performed.

Results:

In 2 cases of the total patient number (54 patients, 56 breasts) no SN-s were detected. (In these cases the axilla was infiltrated on palpation, the preoperative breast US scan gave positive results.) SN-s were examined in 54 cases, giving negative results in 33 cases, while in 21 cases lymph node metastases were confirmed. In 4 of 21 cases metastases were detected in the SN-s exclusively, however SN-s were negative in 7 cases, while mestastases were found in other lymph nodes. That means that metastases of the axillary regional lymph nodes were detected by SN scintigraphy with 67% sensitivity in the patient population examined. Our results are much better, however, if we take into consideration only tumours less than 2.5 cm and exclude cases with lymph node metastases confirmed previously by cytology. Forty cases fall in this category. Histology detected metastases in 9 cases. Eight patients were SN positive and moreover in 3 patients of these metastases were found only in the SN. Other lymph node metastases were disclosed in 1 SN negative patient, but this was a patient who underwent surgery earlier and our examination was performed because of the indication of re-operation for local recidiva. Lymph node metastases were not revealed in 31 patients. In this patient group axillary regional lymph node metastases were detected by SN scintigraphy with 89% sensitivity. (If we take into account that the value of this examination is restricted in already operated breasts, and include only patients with appropriate indication, then the sensitivity amounts to 100%.) The effects of SN isotope labelling on the performance of preoperative breast US scan were also examined. The

re-examined lymph node seemed to be positive in 9 cases during the control US scan in the axilla which was thought to be negative previously. This was actually histologically positive, too, in 3 cases, while in 6 cases there were no metastases in the background. During the first examination the sensitivity was 59%, specificity 79%, and validity 71%; on the other hand during the control US scan, performed after the isotope labelling, sensitivity increased to 69%, while specificity and validity decreased to 60% and 64% respectively.

II Sentinel lymph node scintigraphy with completions

Patients and methods:

Between 10 January 2005 and 12 December 2005 100 female patients were examined in whom malignant breast tumour was confirmed using FNB. The average tumour size was 16 mm (3-30 mm), where the tumour was not palpable in 32 cases. Clinically the axilla was negative in each case. A combined technique was used to label SN. Radiopharmaceutical was administered peritumorally with US guidance (80 MBq 99mTc-Senti-Scint in 0.8 ml) in 4-8 portions. Anterior and lateral planar scans of the chest and axillary region were performed 1 and 3 hours following the injections and the anterior and lateral projections of the SN were marked on the skin. In 71 patients the targeted US scan and FNB of the SN, as well as cytology, were performed on the same day based on the projection marked on the skin. The operation was carried out the next day. Before the operation PB dye was injected periareolarly to mark the lymph vessels and lymph nodes. After finding and removing SN low axillary block dissection was performed, and in lymph node involvement (positive SN cytology or intraoperative examination) total axillary block dissection was carried out. 'Low axillary block dissection' means the removal of the SN and lymph nodes above it and of adipose tissue to the level of the intercostobrachial nerve, which averages 4 (3-6) lymph nodes.

Results:

In 71 patients of the 100 examined cases the preoperative US guided SN FNB and cytology in 5 cases (7%) could not be evaluated. In 44 cases (62%) it was negative and in 10 cases (14%) positive, indicating the metastatic involvement of the SN. However, in 12 cases (17%) the results of cytology were negative even if the hystology of SN detected metastatic lesion. In 5 cases (5%) of 100 observed female patients SN could

not be identified with scintigraphy, in 1 case of these axillary lymph node metastasis was confirmed. In 25 cases (25%) the histology of SN was positive, but in 14 cases (14%) only the SN was positive and in 6 cases (6%) of these the SN was only radioactive, consequently PB negative. In 70 cases the histology of SN was negative, but in 2 cases of these another one was positive. In 16 cases re-operation was needed: in 10 cases due to an affected or small margin and DCIS (ductal carcinoma in situ), in 5 cases due to complete axillary block dissection and in 1 case due to both. In 4 cases of re-operated patients the tumour was not palpable. (Three patients denied additional surgery.) The surgical complications were negligable: in 10 cases seroma developed, in 2 cases after complete axillary block dissection.

Summary, conclusions

1 It can be noted that 99mTc-MDP and 99mTc-MIBI scintimammographies using SPECT are relevant alternatives in the preoperative diagnostics of breast tumours.

2 Althogh the 99mTc-MDP scintimammography showed lower sensitivity and specificity in comparison with 99mTc-MIBI scintimammography in the detection of primary tumours as well as lymph node metastases, the procedure may be useful as a complementary method because of its low price and the ability to detect bone metastases simultaneously, however for substitution of 99mTc-MIBI scintimammography it is not suitable.

3 There is a positive correlation between the intensity of 99mTc-MIBI accumulation and tumour size, as well as tumour vascularisation (number of cross-sections of vessels).

4 Because of its high sensitivity and specificity, 99mTc-MIBI scintimammography is a useful and important complementary method in the preoperative investigation of breast tumours, in the differentiation of malignant or benign tumours, in the assessment of the prognosis, degree of malignity and invasiveness.

5 99mTc-MIBI scintigraphy should be performed in cases of uncertain mammographic and breast US findings, especially in premenopause and in patients with dense breasts.

6 99mTc-MIBI SPECT/CT is a useful and sensitive method in the follow-up of neoadjuvant chemotherapy of breast cancer which combines function and morphology,

in addition to quantitative analyses. The first examination, before the beginning of the therapy, can detect unknown metastases, which modify the therapy.

7 The SN scintigraphy is a suitable and safe method for the detection of axillary lymph node metastases in breast tumours, primarily in cases with smaller (smaller than 2.5 cm) breast tumours and in cases where the axilla is clinically negative, allowing for avoiding axillary block dissection.

8 For the detection of SN the most reliable method seems to be the US guided peritumoral injection of radiopharmaceutical.

9 With periareolar injection of PB and peritumoral injection of Senti-scint the detected lymph nodes occasionally can differ.

10 The US guided peritumoral administration helps in the correct removal of the tumour, especially in nonpalpable tumours.

11 The targeted, preoperative US examination and FNB of SN is a useful completion in the preoperative diagnostics of breast cancer.

12 Using low axillary dissection, false negative cases, caused by skipping metastases, can be avoided. The surgical complications of low axillary dissection are negligible, and are practically the same as in SN biopsy.

Abbreviations

MIBI – methoxy-isobutyl-isonitrile

FDG – fluoro-deoxy-glucose

MDP – methylene-diphosphonate

PgP – P-glycoprotein

MRP – multidrug resistance protein

SN – sentinel lymph node

SPECT – single photon emission computed tomography

CT – computed tomography

MR – magnetic resonance (imaging)

US – ultrasound

ROLL – radioguided occult lesion localisation

FNB – fine needle biopsy

PB – patent blue

Acknowledgements

My sincere thanks to Zoltán Nemessányi M.D., senior assistant professor, former head of our department, for starting me in my career and also for stimulating my interest towards nuclear medicine.

My grateful acknowledgements are due to Professor Katalin Zámbó M.D. for providing continous guidance in introducing the methods and giving me useful advice. Without the inspiration provided by her this thesis could not have been written.

My thanks are also due to dr. Sára Jeges for her indispensable help in processing data for the statistical analysis.

Let me express my gratitude to all my colleagues at the Department of Nuclear Medicine for their assistance in performing the examinations supporting my work. Special thanks to Zoltán Miklovics for his enormous technical assistance.

I would also like to recognise all of my clinician colleagues who have participated in my team implementing the methods, providing me with their data.

Finally my heartfelt thanks to my family for their generous support.